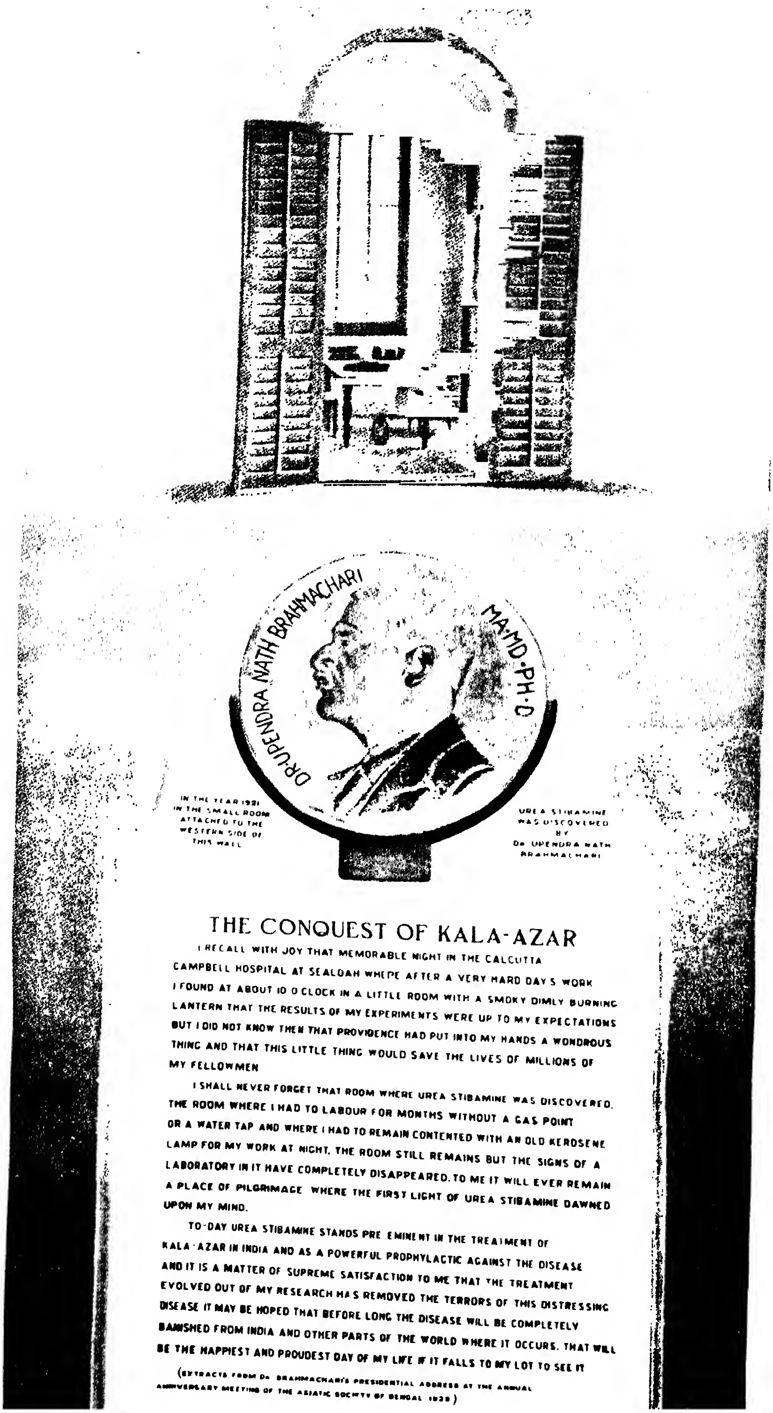
GLEANINGS FROM MY RESEARCHES

VOL. I

KALA-AZAR, ITS CHEMOTHERAPY



DR. UPENDRA NATH BRAHMACHARI M.A. M.D. PH.D
IN THE YEAR 1921 IN THE SMALL ROOM ATTACHED TO THE WESTERN SIDE OF THIS WALL
UREA STIBAMINE WAS DISCOVERED BY DR. UPENDRA NATH BRAHMACHARI
THE CONQUEST OF KALA-AZAR
I RECALL WITH JOY THAT MEMORABLE NIGHT IN THE CALCUTTA CAMPBELL HOSPITAL AT SEALDAH WHERE AFTER A VERY HARD DAY'S WORK I FOUND AT ABOUT 10 O'CLOCK IN A LITTLE ROOM WITH A SMOKY DIMLY BURNING LANTERN THAT THE RESULTS OF MY EXPERIMENTS WERE UP TO MY EXPECTATIONS BUT I DID NOT KNOW THEN THAT PROVIDENCE HAD PUT INTO MY HANDS A WONDROUS THING AND THAT THIS LITTLE THING WOULD SAVE THE LIVES OF MILLIONS OF MY FELLOWMEN
I SHALL NEVER FORGET THAT ROOM WHERE UREA STIBAMINE WAS DISCOVERED, THE ROOM WHERE I HAD TO LABOUR FOR MONTHS WITHOUT A GAS POINT OR A WATER TAP AND WHERE I HAD TO REMAIN CONTENTED WITH AN OLD KEROSENE LAMP FOR MY WORK AT NIGHT. THE ROOM STILL REMAINS BUT THE SIGNS OF A LABORATORY IN IT HAVE COMPLETELY DISAPPEARED. TO ME IT WILL EVER REMAIN A PLACE OF PILGRIMAGE WHERE THE FIRST LIGHT OF UREA STIBAMINE DAWNED UPON MY MIND.
TO-DAY UREA STIBAMINE STANDS PRE-EMINENT IN THE TREATMENT OF KALA-AZAR IN INDIA AND AS A POWERFUL PROPHYLACTIC AGAINST THE DISEASE AND IT IS A MATTER OF SUPREME SATISFACTION TO ME THAT THE TREATMENT EVOLVED OUT OF MY RESEARCH HAS REMOVED THE TERRORS OF THIS DISTRESSING DISEASE. IT MAY BE HOPED THAT BEFORE LONG THE DISEASE WILL BE COMPLETELY BANISHED FROM INDIA AND OTHER PARTS OF THE WORLD WHERE IT OCCURS. THAT WILL BE THE HAPPIEST AND PROUDEST DAY OF MY LIFE IF IT FALLS TO MY LOT TO SEE IT
(EXTRACTS FROM DR. BRAHMACHARI'S PRESIDENTIAL ADDRESS AT THE ANNUAL ANNIVERSARY MEETING OF THE ASIATIC SOCIETY OF BENGAL )

# GLEANINGS FROM MY RESEARCHES

## VOL. I

## KALA-AZAR, ITS CHEMOTHERAPY

BY

SIR UPENDRANATH BRAHMACHARI, KT.,
M.A., M.D., PH.D., F.R.A.S.B., F.N.I.

*Professor of Tropical Medicine, Carmichael Medical College,*
*Professor of Biochemistry, University of Calcutta.*

The *gleaners* spread around, and here and there,
Spike after spike, their scanty harvest pick.
—*Thomson.* Autumn, l.165.

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DEDICATED TO THE MEMORY OF THE POOR MISERABLE SUFFERERS
WHO DIED OF KALA-AZAR BEFORE THE DAYS OF ANTIMONY
AND TO THOSE WHO HAVE BEEN SAVED FROM THE
HORRORS OF THIS DREADFUL DISEASE THROUGH
THE LABOURS OF THE AUTHOR IN THE MIDST
OF TROUBLES AND DIFFICULTIES

## CONTENTS

# FOREWORD

The author's apology for reprinting some of his published papers in a collected form is to infuse the spirit of research into the minds of students of medicine in India and not least amongst those whose paths are restricted to institutions where proper facilities for research are not yet available. These collections also create a sense of happiness in the mind of the author as they remind him of his own past struggles in carrying on his researches. There has been no attempt to observe a logical or even a chronological sequence and the papers are set out in the manner which, it is hoped, is best calculated to provide stimulating and attractive reading.

The first volume of this work contains a series of the author's papers on kala-azar including chemotherapy of antimonial compounds in kala-azar infection which have appeared from time to time in various journals. They record the evolution of his advances in the treatment of a terrible tropical disease. The more ambitious student will find some portion of the subject dealt with at greater length in the author's previous works on kala-azar, such as (*1*) *Kala-azar: Its Treatment* (*Butterworth & Co., Calcutta, 1917*) and subsequent editions, (*2*) *Treatise on Kala-azar* in German in *Professor Dr. Carl Mense's Handbuch der Tropenkrankheiten. Vol. IV, 1926* and (*3*) *A Treatise on Kala-azar* (*John Bale Sons & Danielsson, Ltd., London, 1928*). In these will be found many conclusions which the author has formed after continued research, but which have not been the subject-matter of previous articles and are therefore omitted from the present work. The last chapter in this volume, *The Conquest of Kala-azar*, is a portion

of the author's Presidential Address at the Medical Section of the Indian Science Congress, Twenty-fifth Session, 1938.

The second volume contains the author's papers on malaria including his *studies on the chemistry and chemotherapy of quinoline and acridine compounds which resulted in the synthesis of a compound allied to atebrin.* The first chapter in this volume is a continuation of the author's Presidential Address, referred to above. It consists of certain observations on the chemotherapy of malaria.

It is intended that the author's other published papers will form the subject matter of another volume.

Some of the articles in these volumes have been edited by the writer.

The author hopes that a time may come when his past struggles in research will see the light of day.

# LIST OF ILLUSTRATIONS

## *Plates*

*Diagrams*



*Temperature Charts*

Facing Page

[Reprinted from the Indian Medical Gazette, Vol. LVII, No. 4, April, 1922]

# A NEW FORM OF CUTANEOUS LEISHMANIASIS—DERMAL LEISHMANOID

The following paper on "A New Form of Cutaneous Leishmaniasis" was read by me at the meeting of the Medical Section of the Asiatic Society of Bengal held on 8th February, 1922.

The various forms of cutaneous and muco-cutaneous leishmaniasis are divided by Castellani and Chalmers as follows :—

(1) Cutaneous.
(2) Muco-cutaneous.
(3) Oro-pharyngeal.

The cutaneous forms are divided by them into :—

(*a*) The common variety—The oriental sore.
(*b*) The verrucose variety.
(*c*) The keloid-form.
(*d*) The frambœsiform.
(*e*) The papillomatous variety.
(*f*) The deep ulcerative variety.

Laveran describes the following forms of cutaneous leishmaniasis :—

(*A*) The oriental sore.
(*B*) American leishmaniasis : (*a*) the cutaneous ulcerating form, (*b*) the cutaneous non-ulcerating form which may be either papillomatous or macro-tuberculous.

The variety of cutaneous leishmaniasis described in the present paper is of extreme pathological and clinical importance. It differs from any form of cutaneous leishmaniasis described in literature and appears to afford the missing link between cutaneous and visceral leishmaniasis or kala-azar and leads one to conclude that the special pathogenic properties of the parasites of kala-azar may be so modified after antimonial treatment that it may subsequently give rise clinically to a form of cutaneous leishmaniasis, thus proving the identity of the parasite of kala-azar and that of cutaneous leishmaniasis.

Among the multitude of kala-azar patients treated by me with intravenous injection of antimony, I met with four cases which, within six months to two years after completion of treatment, came to me with a peculiar form of cutaneous eruptions which, at first sight, gave an impression of tuberculous leprosy. In none of them, however, could any lepra bacilli be found. When they came to me with these eruptions, there were no clinical symptoms of kala-azar.

The appearance of these cutaneous eruptions in patients who have apparently recovered from kala-azar after antimonial treatment made me suspect that they might be due to a cutaneous infection of these individuals in whom there was not a complete sterilization of the organs against the leishmania, though their virus had been attenuated by repeated antimonial injections. This led me to examine the scrapings from the cutaneous nodules of these cases with the help of Dr. Surendra Nath Ghose, Bacteriologist, Presidency General Hospital, Calcutta. The examination of the scrapings led to the remarkable discovery that the eruptions were due to cutaneous infection by the parasites of kala-azar.

During the antimonial treatment of kala-azar, the following results may follow :—

(1) Cure.
(2) Apparent cure followed by a relapse.
(3) No improvement.

A fourth result may follow, and this is what happened in the four cases mentioned above. The visceral leishmaniasis may be cured, but a few leishmania may be left behind with their virus so attenuated that they gave rise to a milder disease, namely, cutaneous leishmaniasis.

I give here the full history of the last case in which this transformation of a case of visceral leishmaniasis (kala-azar) into one of cutaneous leishmaniasis took place.

Patient, æt. 31, an inhabitant of Barisal, gave a history of fever coming on with rigors from February, 1917, which was not benefited by quinine. In May, 1917, he had an attack of pneumonia. His fever persisted and there was progressive enlargement of the spleen. He was again treated with quinine which was given intramuscularly in doses of 10 grains for 6 days. He states that after this he was free from fever till the end of June, 1917. In July, he again had an attack of intermittent fever, the temperature ranging between 99° F. and 105° F. He was again given intramuscular injections of quinine but with no benefit.

In January, 1918, he came to Calcutta for the treatment of persistent fever with the enlargement of the spleen and the liver. The spleen extended 6 inches below the costal margin and the liver extended 3 inches below the costal arch. The fever was of an intermittent type. He was at first given a course of treatment with soamin. The results of blood examination before treatment with soamin were R. B. C. 3,000,000, W. B. C. 3,500, Hb. 30 per cent. and differential count showed polymorphonuclears 60 per cent., lymphocytes 24 per cent., large mononuclears 14·8 per cent., and eosinophiles 1·2 per cent.

The treatment with soamin was not followed by any improvement. Spleen puncture was made and the smear showed the presence of Leishman-Donovan bodies. A few L. D. bodies were also found in peripheral blood. The patient was now treated with intravenous injection of tartar emetic given twice a week in doses of $\frac{1}{2}$ to 10 c.c. He had altogether thirty injections. The fever stopped after 10 injections. When he left the treatment, there was marked improvement in his general condition, the spleen and the liver could not be felt below the costal margin and the blood condition was :—

R. B. C. 4,000,000. W. B. C. 7,500. Hb. 70 per cent.

No parasites could be found on spleen puncture.

He has had no fever since his treatment with antimony was stopped.

In the beginning of 1919, he noticed faint whitish patches on his face. These gradually spread. These patches were neither anæsthetic nor hyperæsthetic. They gradually spread over the whole body in front and behind in about six months. He was at first treated with arsenic internally. The patches became worse during cold weather. Subsequently, papillomatous nodules appeared over the face, the trunk and the extremities.

Patient was seen by me very recently. I asked Dr. Ghose to make a very careful examination of the scrapings and the juice from the papillomatous nodules for the presence of L. D. bodies. The smears showed a very large number of L. D. bodies in some of the slides.

*Description of the present rash.*—The whole of the body is covered with eruptions which are described as follows :—

(1) On the face there are papillomatous nodules somewhat resembling small leprotic nodules.

(2) There is a slight erythematous appearance on the cheeks and the forehead.

(3) On the trunk, the upper and the lower extremities, there are slightly raised brown patches which are extensively spread over the whole body. A few papules are also present in these parts.

(4) There are some erythematous patches in the extremities, especially the lower.

(5) No ulceration or scab formation in any part of the body. Other features—no anæsthesia, no loss of knee-jerks, no thickening of the nerves. No eruptions in the mucous membrane of the mouth and nostrils.

Liver and spleen normal. On examination of the splenic blood by spleen puncture, no L. D. bodies were found. No rise of temperature. The patient complains of no other trouble, except the ugly appearance of the body due to the eruptions.

Result of blood examination on 1st February, 1922 :—

Hb. 75 per cent.
R. B. C. 4,500,000.
W. B. C. 10,000.
Polymorphonuclears 62 per cent.
Lymphocytes 24 per cent.
Large mononuclears 6 per cent.
Eosinophiles 8 per cent.

The blood report does not at all correspond to that of kala-azar. No L. D. bodies could be detected in the peripheral blood.

*Examination of the scrapings.*—L. D. bodies are found in very large numbers, especially in the juice expressed from the papillomatous nodules. A few have also been found from the brownish patches. No lepra bacilli.

In view of the fact that the eruptions are due to leishmania infection whose virus has been modified by antimonial treatment, I propose to call this form of cutaneous leishmaniasis *dermal leishmanoid* just as small-pox modified by vaccination is called varioloid.

I shall study the morphological character of the flagellate forms of these parasites after culturing them with the help of Major Knowles, I.M.S., Protozoologist, Calcutta School of Tropical Medicine.

This case, along with three others of a similar type that I have observed, is a remarkable one, as they appear to point to the identity of the parasites of visceral and cutaneous leishmaniasis.

It seems that the virus of the parasite of kala-azar was attenuated in these cases by the antimonial treatment and a case of deadly visceral leishmaniasis was converted into one of cutaneous leishmaniasis. We thus have a direct proof of the identity of the parasites of visceral and dermal leishmaniasis, which has been attempted to be proved indirectly by complicated inoculation experiments.

Of the three other cases met with by me, one resembled the present case, the rash being generalized over the whole body. The other two cases had less generalized rash, most of the papillomatous eruptions being present on the face, there being some brownish patches over the arms.

One of these cases was treated with further injections of antimony and he appeared to improve. The second one, a boy of 15 years, was given six intravenous injections of tartar emetic in doses of 3 to 5 c.c., but he left treatment before any improvement was noticed. I propose to treat the present case with combined treatment of intravenous injection of antimony and soamin and shall report the results in a future communication.

It has been suggested by Manson that the treatment of kala-azar with a vaccine made from the virus of oriental sore is worth trial. May it be further suggested that in places where kala-azar is very prevalent, the inhabitants should be vaccinated with the virus of oriental sore as a prophylaxis against kala-azar ?

Apart from the interest in the above case on account of its forming a new hitherto unknown clinical entity, it raises the following most suggestive questions :—

(1) Are the parasites of kala-azar in the process of destruction by antimonial treatment eliminated by the skin and are cases of kala-azar therefore more infective during antimonial treatment ?

(2) If the parasites are eliminated by the skin, do they also enter the system through the skin at the time of primary infection ?

The above case, after being exhibited by me at the meeting of the Medical Section of the Asiatic Society of Bengal, held on 8th February, 1922, was exhibited at the Calcutta School of Tropical Medicine on 9th February, 1922.

I append here a drawing showing the eruptions on the upper part of the patient's body. A drawing from the scrapings from one of the nodules is also appended herewith showing the presence of *Leishmania donovani* which mostly seems to be extra-corpuscular in the smear. As stated before, I have met with four cases of dermal leishmanoid.

Perhaps such cases are more common than has been suspected and more cases will be met with by observers who are treating kala-azar with antimonial preparations.

I am indebted to the Editor, *Indian Medical Gazette*, for announcing my discovery of this new form of cutaneous leishmaniasis in the *Indian Medical Gazette* for March, 1922.

I suggest that workers in the field of kala-azar should look out for such cases of infection by *Leishmania donovani* *sine* kala-azar as a result of antimonial treatment.

Since the above paper was sent to the editor, *Indian Medical Gazette*, I have succeeded in developing flagellated forms of *Leishmania donovani* with the help of Major R. Knowles, I.M.S., on N.N.N. medium from the juice obtained from the eruptions by puncture. Blood cultures were negative.

[Reprinted from the Indian Medical Gazette, Vol. LVII, No. 4, April, 1922]

# A NOTE ON A NEW DISEASE—" DERMAL LEISHMANIASIS " (BRAHMACHARI)

By J. W. D. MEGAW, LIEUTENANT-COLONEL, I.M.S.

The above paper on " dermal leishmanoid " by Dr. Brahmachari is of very exceptional interest. It records a disease hitherto unknown to science, but what is more remarkable is that the disease appears to have been produced by human agency. As the writer of this note remarked when Dr. Brahmachari showed one of the cases at the Calcutta School of Tropical Medicine, it is a unique experience for a medical man to be the agency through which a new disease is produced.

The disease differs entirely from drug eruptions in being a specific parasitic affection which follows the administration of antimony salts by the intravenous route.

It is possible that the disease may occur under other conditions but there is no evidence that this takes place, and it is not likely that a dermal leishmaniasis of so distinct a kind would have escaped notice hitherto, considering the number of practitioners who are in the habit of examining scrapings in all doubtful cases of chronic skin disease. Those who have seen the case shown by Dr. Brahmachari, agree that the disease is a hitherto unknown form of leishmaniasis and Dr. Brahmachari is to be heartily congratulated on the very interesting and important discovery which he has made.

There will be differences of opinion as to the interpretation of the findings. It has not yet been proved that the cutaneous leishmaniasis is due to a modified virus.

There is some factor which has caused the parasite to behave in so remarkable a manner, but it remains to be seen whether the virus is changed in its nature. Indeed the very example which is given by Dr. Brahmachari, *viz.*, varioloid, is really an example of virus whose nature is not essentially changed. It is rather the human body which reacts differently in cases of varioloid and the same thing probably happens in the case of the dermal leishmaniasis under discussion.

There is, however, an important difference in that the parasite in dermal leishmaniasis produces manifestations which are essentially different in type from those of kala-azar of which it is an after-development. It is also impossible to agree with Dr. Brahmachari when he says that the cases "appear to point to the identity of the parasites of visceral and cutaneous leishmaniasis." What the cases point to is the identity of the parasites of this particular form of dermal leishmaniasis with those of kala-azar, but it does not follow that the parasites of other forms of leishmaniasis, such as oriental sore, are identical.

The parasites from one of Dr. Brahmachari's cases have been cultivated by Major Knowles, and, as would be expected, the cultures appear to be identical with those of the Leishman-Donovan body, and up till the present there is no evidence that they are in any way different from that parasite. Meantime it is to be hoped that other workers will look out for further cases of this disease, especially in Assam, where thousands of cases of kala-azar have undergone treatment by antimony preparations.

A look-out should of course be kept for possible cases arising independently of antimony treatment.

Already another case of the disease has been recognised by my assistant, Dr. Bhattacherji, in a patient who had undergone antimony treatment.

The name proposed by Dr. Brahmachari does not seem to be suitable, the disease is a leishmaniasis, not a leishmanoid.

The name which appears to me to be most appropriate has the serious drawback of being clumsy, it is "Post-Antimonial Dermal Leishmaniasis" (Brahmachari) or more briefly "Brahmachari's Dermal Leishmaniasis." The after-history of the cases will be watched with the greatest interest, and meantime there will doubtless be a good deal of speculation as to the reason for the production of the disease.

Have the parasites in the accessible parts of the circulatory system been killed off ? And have those which remain in the less accessible parts of the circulatory system been cut off from the destructive action of the antimony and the blood by an obliteration of the capillaries as a result of the action of the antimony ? If such an action results from the antimony injections, it is possible that it is a question of the parasites being entrapped in places where they are capable of growing unhindered by whatever immunising mechanism there is, and that a general immunity against the parasites has no chance of developing, because the parasites in the body in general have been killed off by the antimony.

It now remains to be seen whether a course of antimony treatment will influence the skin manifestations and also whether the disease will remain localised in the skin.

If any of the cases refuse to undergo antimony treatment there will also be an opportunity of seeing the natural evolution of the disease.

[*N.B.*—Subsequent history of this case is described in the *Transactions of the Royal Society of Tropical Medicine*, Vol. XXIII, No. 3, pp. 301-04, issued November, 1929.
—*Editor.*]

[Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922]

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, January 3, 1922]

## PART I

THE TOXICITY OF ANTIMONYL TARTRATES—THE INFLUENCE OF THE BASIC RADICLE OF AN ANTIMONYL TARTRATE UPON ITS TOXICITY—SOME ARYL PENTAVALENT ANTIMONIAL COMPOUNDS—P-AMINO-PHENYL STIBINIC ACID AND SOME OF ITS DERIVATIVES—THEIR TOXICITY—THE THERAPEUTIC VALUE OF AMMONIUM ANTIMONYL TARTRATE AND UREA STIBAMINE

### *The Symptoms of Antimony Poisoning in the Guinea-pig and the Rat after Injection of Antimonyl Tartrates*

The symptoms of acute antimony poisoning in the guinea-pig and the white rat are not always constant. Vomiting and purging which are frequently observed in man after intravenous injections are not at all common in guinea-pigs even after toxic doses, and it is not rare to find solid fæces in the large intestines even when the animal dies of severe acute symptoms.

The symptom-complex of the intoxication produced by the various antimonyl tartrates enumerated below does not vary much whatever may be the salt used. They are divisible into two groups of phenomena :—

(1) Nervous. (2) Nutritional.

In some severe cases of acute poisoning, the animal passes into a state of prostration with complete paralysis of the central nervous system within $\frac{1}{2}$ to 1 hour after injection. In less severe cases the symptoms come on more gradually. Marked tremor and chattering of the teeth are sometimes very characteristic features of intoxication and then the animal passes into a state of coma and in the comatose state may develop spasmodic movements of groups of muscles coming on at intervals of $\frac{1}{2}$ to 1 minute. In fatal cases the breathing is hurried and pulse quick. In some cases, the animal lies in a comatose condition for some hours before death. Sometimes the animal shows marked muscular tremors and incoordination when disturbed. Salivation has been observed in many cases but is not a constant symptom. In some cases a few minutes after intravenous injection the animal exhibits a very marked spasmodic movement of the whole body at frequent intervals. In some cases spasmodic contraction of diaphragm resembling hiccough in man has been observed. In many cases, soon after the injection of a fairly large dose, the animal frequently scratches its mouth with its front legs. Sometimes even after sublethal doses the animal appears to be ill and faint for a short time. It is unsteady, the gait is staggering and the animal may roll about. The animal is less active and takes its food less freely than usual. If it survives for 10 or 12 hours after the injection, then there is development of a peculiar bloated appearance of the face in fatal cases. In cases that survive, this phenomenon is slightly or not at all marked.

There may be marked emaciation in cases that survive 2 or 3 days after injection but frequently the animal regains in weight in a week's time.

In some cases, the animal progressively loses in weight. It takes very little food, remains dull and dies on the 7th or 8th day. Such cases are rare and generally it may be stated that if recovery takes place, it comes on within two or three days and is complete.

### *Period at which Death Takes Place after Doses within the Toxic Range*

The earliest period at which death took place in guinea-pigs after minimum lethal doses was 4 hours. In some cases, the animals took 12 to 18 hours to die after injection of the minimum lethal doses. With smaller doses, but still within the toxic range, the animals, that did not survive, died 24 to 36 hours after injection. Sometimes the animals survived for 8 to 10 days, and after death they showed symptoms of antimony poisoning. Cases of delayed antimony poisoning will be described in another series.

## Pathology of the Intoxication

The pathological changes produced in the animal may be studied under the following heads :—

(1) Local effects.

(2) Systemic effects.

It may be stated that, generally speaking, the local effects produced after intramuscular injections of the antimonyl tartrates are much less marked in the case of the guinea-pig and the rat than in the case of man. There may be some irritation and swelling at the seat of injection but necrosis or destruction of tissues is rarely met with—a phenomenon frequently met with in the case of man.

### *Systemic Effects*

The effects produced by the antimonyl tartrates upon the animal as a whole, when given in toxic doses, are very marked. In general, after toxic doses, the pathological lesions consist of hæmorrhages into the internal organs and necrosis of their cellular elements. The organs in which the changes are most marked are (1) Lungs, (2) Kidneys and (3) Liver. Marked changes in the gastro-intestinal tract are

not frequently met with. Sometimes there is congestion of or even hæmorrhage or ulceration in the gastric mucosa. There may be hæmorrhages into the substance of the spleen. Among the ductless glands that have been studied marked pathological changes may take place in the adrenals and pituitary. No change has been observed in the thyroid.

I shall now describe these changes in the different organs in detail :—

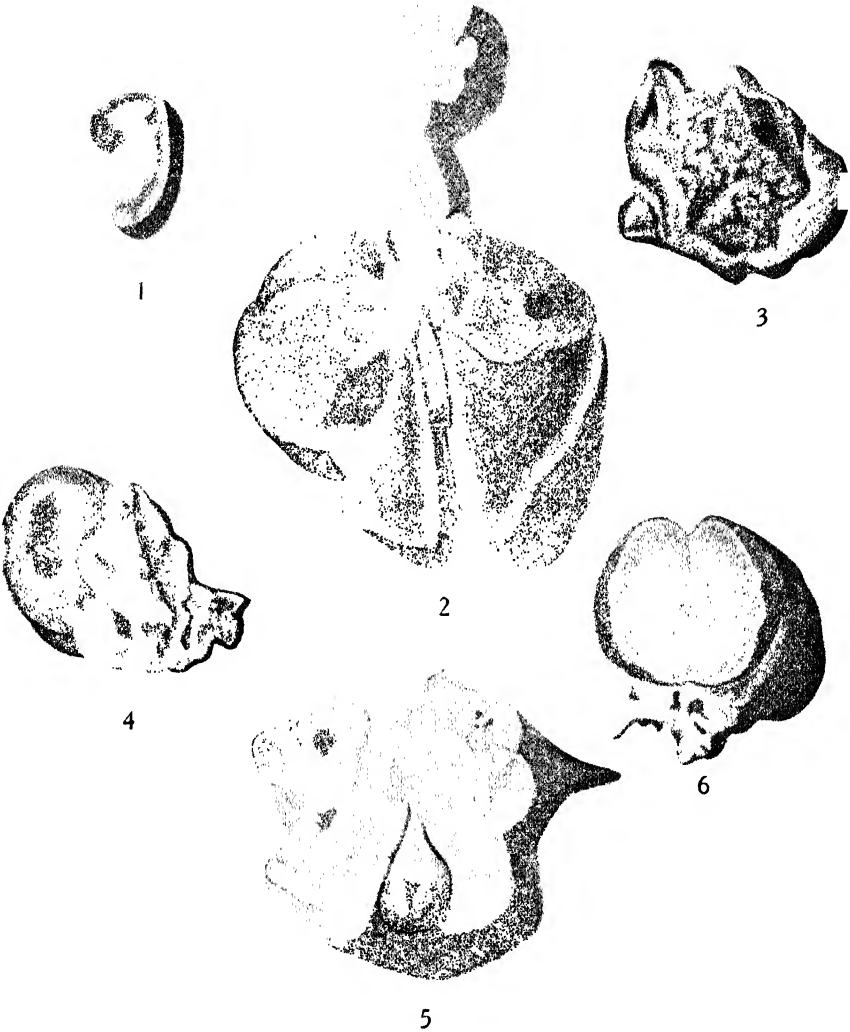
(1) *Lungs.*—In about 90 per cent. of the cases that die of acute poisoning, the whole lungs are in a state of extreme congestion with hæmorrhages into their substance and alveoli. On section, blood pours out freely from the cut surfaces. On microscopic examination, extensive hæmorrhages into the substance of the lungs with destruction of the parenchyma with round-celled infiltration and exudation of necrosed material into the alveoli of the lungs are met with. (See Plates XII, XIII and XIV.) In one animal there was evidence of lobar pneumonia in one lung, but this might have been accidental.

(2) *Kidneys.*—In fatal cases, marked destructive changes are met with in the kidneys. In acute cases, the kidneys are slightly enlarged. There may be hæmorrhages into their capsules. The congestion is sometimes most marked in the boundary zone and frequently extends outwards along the medullary rays towards the capsular surface. Sometimes there is cloudy swelling and sometimes necrosis of the kidney epithelium. Hæmorrhage which may sometimes be very extensive may be seen in the interstitial tissues of the kidney. The tubules of the kidneys may be blocked with granular debris. (See Plates XII and XV.)

(3) *Liver.*—In some cases, the liver presents a pale, yellowish appearance which is indicative of extensive fatty change. On the surface there may be spots of hæmorrhage, sometimes very extensive. In other cases the liver presents a deeply congested appearance with hæmorrhages into its

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922*]

PLATE XII

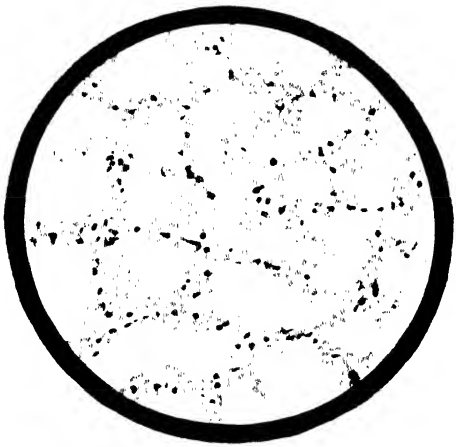



1—SPLEEN
2—LUNGS
3—STOMACH [opened]
4—KIDNEY
5—GALL BLADDER
6—KIDNEY [opened]

The different organs cut open showing extensive hæmorrhages into their substance brought about by antimony poisoning.

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922*]

PLATE XIII



Section of lung showing hæmorrhage into the interstitial tissue.

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922*]

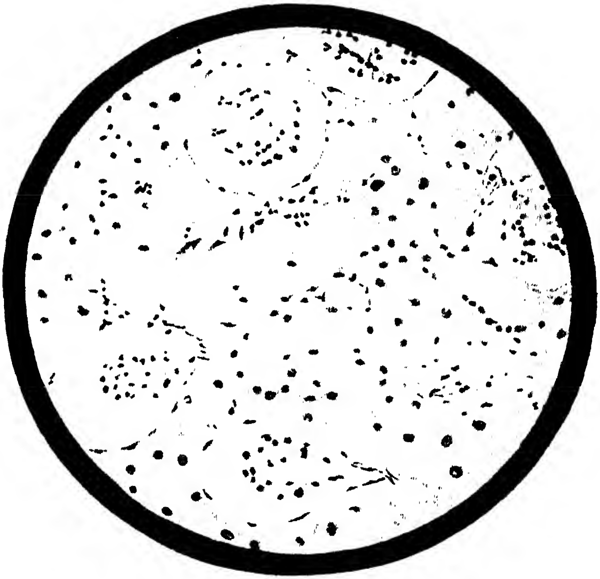
PLATE XIV

Section of lung tissue showing hæmorrhage round cell infiltration and blocking of alveoli with debris.

Serial No. 4

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922*]

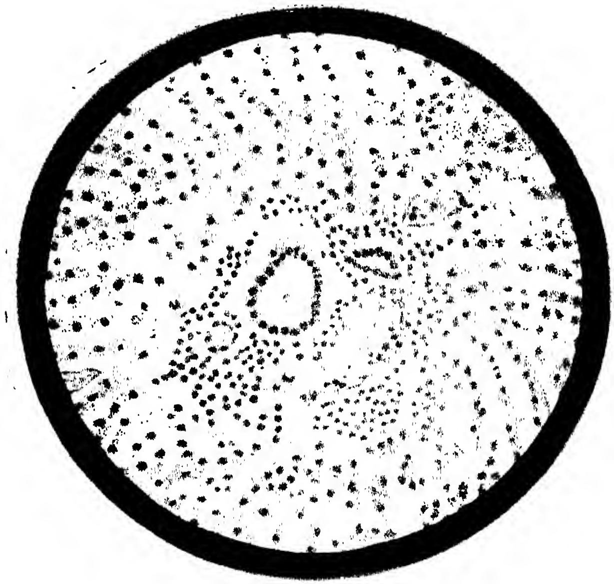
PLATE XV



Section of kidney showing hæmorrhage into the interstitial tissue, cloudy swelling and destruction of the kidney epithelium, and exudation of granular material into the kidney tubules.

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922*]

PLATE XVI

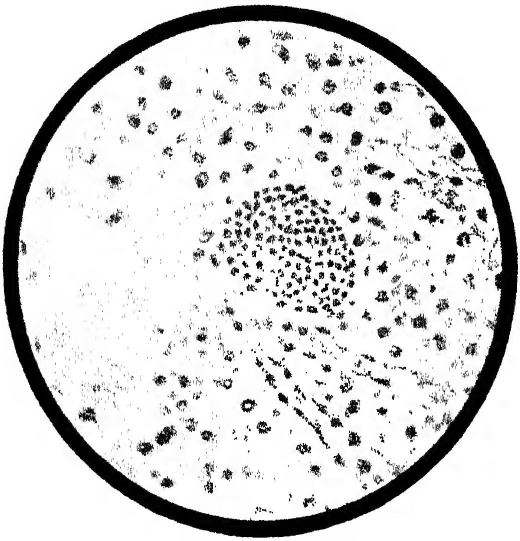


Section of Liver showing round infiltration round the portal system, fatty degeneration, cloudy swelling and hæmorrhage.

Serial No. 6

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No 2, October, 1922*]

PLATE XVII



Section of Spleen showing destruction of spleen pulp.

substance. The latter is observed in those cases in which the animal dies within a few hours after injection and the former when the animal dies after more than 24 hours after injection. There may be hæmorrhages into the substance of the gall bladder. Bile may be blood-stained. (See Plate XII.)

On microscopic examination, the following changes are noticeable :—(See Plate XVI.)

(*a*) Round-celled infiltration around the portal system and hæmorrhage into the interstitial tissue.

(*b*) Necrosis and extensive fatty degeneration of the hepatic cells.

(*c*) Blocking of bile capillaries with granular debris.

(4) *Spleen*.—There may be hæmorrhages into the substance of the spleen. Necrosis of the splenic pulp may be observed. (See Plate XVII.)

(5) *Gastro-intestinal Tract*.—In some cases there may be signs of acute congestion with patches of ulceration in the stomach, but this is not constantly met with. Sometimes the small intestines are deeply congested and there may be hæmorrhages into their peritoneal coating. The large intestines frequently escape and there may be solid fæces inside them.

(6) *Salivary Glands*.—There may be extensive destruction of the secreting cells of the parotids with hæmorrhage and round-celled infiltration. (Plate XXII.)

(7) *Ductless Glands*.—(*a*) *Thyroid*. No changes have been observed in the thyroid.

(*b*) *Adrenals*. Hæmorrhages into the substance of the adrenals are not infrequently observed in the acute cases. The cortical vessels may be swollen. There may be marked

decrease in the cortical pigmentation. Degenerative changes may be observed in the cortex and medulla. (Plate XVIII.)

(c) *Pituitary*. The changes in the pituitary may be divided into two classes :—(See Plates XIX, XX and XXI).

(*i*) Changes that take place in the gland after death from severe acute poisoning.

(*ii*) Changes that take place in the gland after death from subacute poisoning.

In the former, there may be hæmorrhages into the substance of the anterior portion of the pituitary with diminution in the eosinophile staining of the cells. In the latter, marked increase in the basophile staining of the cells with slight hæmorrhages is the characteristic change and there may be shrinking of the protoplasm and the nuclei of the cells with prominence of the interstitial tissue. The same changes may be more or less present in the posterior portion of the pituitary.

## *The Toxicity of Antimonyl Tartrates—The Influence of the Basic Radicle of an Antimonyl Tartrate upon its Toxicity*

Since the discovery of antimony as a specific in the treatment of leishmaniasis, no systematic work has been done to determine the toxicity of the antimonyl tartrates. It is at the same time evident that such an investigation should be of the highest importance as the antimonyl tartrates are the compounds that are still most commonly used in the treatment of the various forms of leishmaniasis.

In the present paper, the toxicity of the following antimonyl tartrates has been investigated :—

(1) Ammonium antimonyl tartrate, (2) Urea antimonyl tartrate, (3) Aniline antimonyl tartrate, (4) Potassium antimonyl tartrate, (5) Sodium antimonyl tartrate.

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922*]

PLATE XVIII

Section of Suprarenal gland showing hæmorrhage in zona reticularies and medulla.

Serial No. 8

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922*]

PLATE XIX

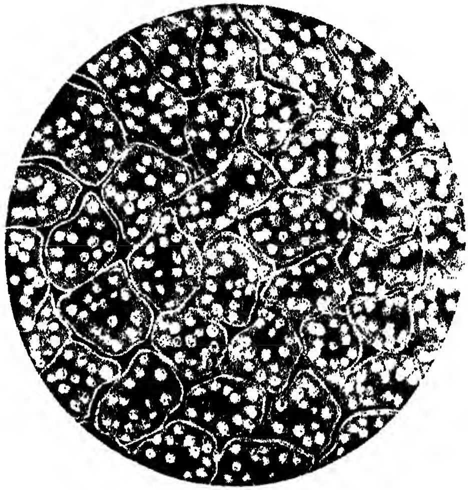


FIG. 1

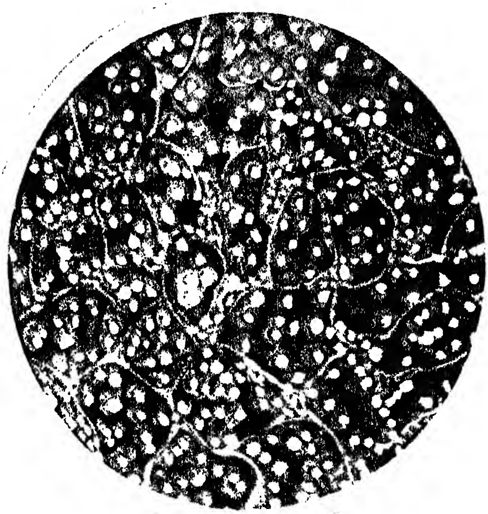


FIG. 2

FIG. 1.—Section of Pituitary, pars anterior, normal

FIG. 2.—Section of Pituitary, pars anterior, showing diminution of eosinophile staining, contracted appearance of cells and hæmorrhage. Death 12 hours after tartar emetic injection.

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922*]

PLATE XX

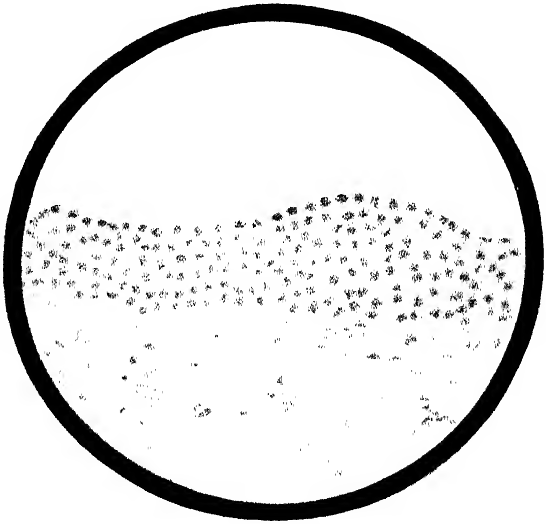


FIG. 1

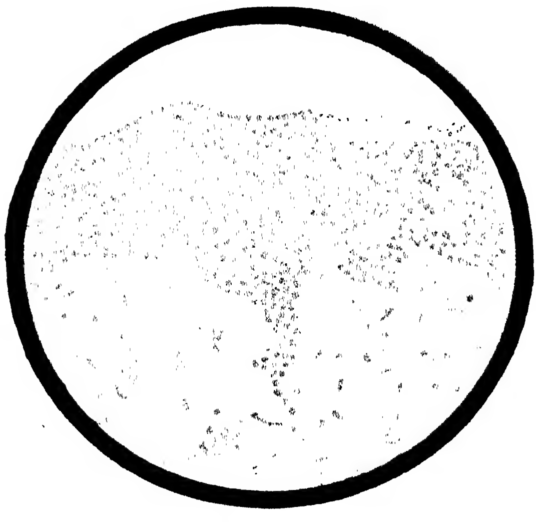


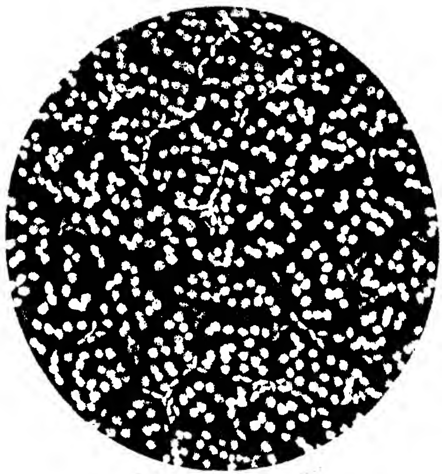
FIG. 2

FIG. 1.—Section of Pituitary, pars posterior, normal.

FIG. 2.—Section of Pituitary, pars posterior. Death from tartar emetic poisoning, the animal died seven days after injection. The section shows marked diminution of eosinophile staining of the cells, nuclei of the cells contracted.

[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1923*]

PLATE XXI



Section of Pituitary, pars anterior, showing marked diminution eosinophile staining of the cells, the nuclei of the cells contracted, and interstitial hæmorrhage. Death took place seven days after emetic injection.

## UREA ANTIMONYL TARTRATE

In the process of my chemotherapeutic investigations, I have succeeded in preparing a new antimonyl tartrate, the urea antimonyl tartrate, the preparation and properties of which have been described by me in the *Journal and Proceedings, Asiatic Society of Bengal* (New Series, Vol. XVI, 1920, No. 8). The therapeutic value of this antimonyl compound in kala-azar has been recorded by me in the *Journal of Tropical Medicine and Hygiene*, August 15, 1921. Since then, with the help of one of my assistants, I succeeded in preparing this compound in another way, which is described as follows :—

1 gram of urea is gently heated with an aqueous solution of 5 grams of tartaric acid for about half an hour. This gives a solution of acid urea tartrate which is subsequently concentrated by gentle heating. To the concentrated solution of the acid urea tartrate, a small weighed quantity of $Sb_2O_3$ is added and the mixture gently boiled till the $Sb_2O_3$ goes into solution. This process is repeated till 4·8 grams of $Sb_2O_3$ are dissolved. The solution is then filtered and concentrated to a syrupy consistency and then allowed to crystallize. In 24 to 48 hours, beautiful crystals separate which are removed and dried on a porous plate and purified by repeated crystallization. Yield = 8 grams.

The salt originally prepared by me corresponded to the following formula :— $CO(NH_2)_2.\ C_4H_5SbO.\ O_6.\ 5H_2O$. Prepared in the above way it contains $\frac{1}{2}H_2O$ as water of crystallization.

### COMPOSITION

Calculated for $CO(NH_2)_2.(C_4H_5SbO.O_6)_2,\frac{1}{2}H_2O$

Sb = 37·55%, N = 4·38%, C = 16·9%, H = 2.34%.

Found Sb = 37·55%, N = 4·28%, C = 16·8%, H = 2.2%.

It thus appears that in urea antimonyl tartrate, urea combines with two equivalents of antimonyl tartaric acid, being therefore different from other salts of urea, in which only one of the amino groups in urea is neutralized by the carbonyl group.

## Ammonium Antimonyl Tartrate

It is best prepared by the interaction of acid ammonium tartrate with $Sb_2O_3$.

6·7 grams of acid ammonium tartrate mixed with 5·8 grams of $Sb_2O_3$ are digested with about 50 c.c. of water till all the $Sb_2O_3$ goes into solution. The solution is filtered and concentrated gently on the water bath. On cooling, crystals of ammonium antimonyl tartrate separate. Yield = 11 grams. The salt is purified by repeated crystallization. It contains $\frac{1}{2}$ molecule of water of crystallization and its antimony content = 38·58% on theoretical calculation. Found Sb = 38·1%.

## Aniline Antimonyl Tartrate

It is best prepared by heating two gram-molecular weights of acid aniline tartrate and one gram-molecular weight of $Sb_2O_3$ in the presence of water.

7·5 grams of tartaric acid are dissolved in water. 4·7 grams of aniline are added to this and the mixture boiled for a quarter of an hour. The solution is filtered and crystallized yielding 10 grams of acid aniline tartrate. 4·9 grams of acid aniline tartrate are digested with 2·9 grams of antimony trioxide in the presence of water, till all the antimony trioxide goes into solution. The solution is then allowed to crystallize. Yield = 5·1 grams.

## Composition

Calculated for $C_6H_5NH_2.C_4H_5SbO.O_6$, Sb = 31·75%.
Found Sb = 31·75%.

It has been prepared in other ways by previous workers which need not be described here.

*Purity of the salts used*:—

(1) The sodium and potassium antimonyl tartrates were specially prepared for me as chemically pure by Messrs. Martindale & Co.

(2) The ammonium, urea and aniline antimonyl tartrates were prepared and purified in my laboratory by repeated crystallization.

*The Antimony Content of the Salts Used, as Estimated by Actual Calculation*

(1) Ammonium antimonyl tartrate, $NH_4.C_4H_4.SbO.O_6.\frac{1}{2}H_2O$, Sb = 38·1%.

(2) Urea antimonyl tartrate, $CO(NH_2)_2.(C_4H_5\,SbO.O_6)_2.\frac{1}{2}H_2O$, Sb = 37·55%.

(3) Aniline antimonyl tartrate, $C_6H_5NH_2,C_4H_5.SbO.O_6$, Sb = 31·75%.

(4) Tartar emetic, $KC_4H_4SbO.O_6.\frac{1}{2}H_2O$, Sb = 36·1%.

(5) Sodium antimonyl tartrate, $NaC_4H_4SbO.O_6.\ 2\frac{1}{2}H_2O$, Sb = 34·1%.*

---

* The following notes are quoted from Wenyon's '*Leishmaniasis: A Review of Recent Literature*" published in *Tropical Diseases Bulletin*, Vol. 19, No. 1, 1922:—

" STIBACETIN.—Sodium paraacetylaminophenylstibinate, $C_{24}H_{25}O_{10}N_3Sb_3Na$, contains theoretically 40·12 per cent. of antimony. The form of 'Stibacetin' sold as Stibenyl in this country in May, 1920, contained 34·85 per cent. of antimony, and that now on sale, which is advertised as Von Heyden's, contains 33·16 per cent. The different batches contained 32·54 and 33·79 = mean 33·16. Sodium antimonyl tartrate, $C_4H_4O_7\,SbNa$, $\frac{1}{2}$ $H_2O$, contains 38·06 per cent. of antimony, and the commercial salt as made for use in medicine is pure Potassium antimonyl tartrate, $C_4H_4O_7Sb\,K.\ \frac{1}{2}\ H_2O$, contains 36·14 per cent. of antimony, and the commercial salt is pure.

All the foregoing percentages are expressed as percentages of metallic antimony for the formulæ as given, including water of crystallization where shown in the formulæ."

.The formula given above for stibacetin corresponds to a compound consisting

*Lethal doses* :—

In the following tables and the subsequent portions of this paper the abbreviations used are explained as follows :—

(1) M. L. D., the minimum lethal dose, *i.e.*, the minimum dose in grams per kilo of body weight which killed all the animals used.

(2) Maj. L. D., the majority lethal dose, *i.e.*, the dose in grams per kilo of body weight which killed 66 per cent only of animals used.

(3) M. T. D., the maximum tolerated dose, *i.e.*, the maximum dose in grams per kilo of body weight which was tolerated by all the animals used.

(4) Maj. T. D., the majority tolerated dose, *i.e.*, dose in grams per kilo of body weight which was tolerated by only 66 per cent of the animals used.

(5) T. R. toxic range, *i.e.*, the range between the mini mum lethal dose and the maximum tolerated dose.

## EXPERIMENTS ON GUINEA-PIGS

### *Method of Administration and Measurement of Doses*

The toxicity experiments on guinea-pigs with the above compounds will be first described in the present paper. The drugs were administered intramuscularly, the injections being given in the outer part of the thigh. The strength of the solution was two per cent in distilled water. In all these experiments, each time the solution was freshly prepared and

of condensation of one molecule of $C_8H_9O_4N$ SbNa. and two molecules of $C_8H_{10}O_4NSb$, 2 $H_2O$. The percentage of antimony present in the compound prepared in our laboratory corresponds to the formula $C_8H_9O_4N$ SbNa, the exact analogue of atoxyl without any water of crystallization. Further observations on this subject will be made later on.

The difference in the antimony contents of some of the antimonyl tartrates as estimated in my laboratory, from those quoted above, is due to their containing different molecules of water of crystallization. It is a well-known fact that the water of crystallization may vary in an antimonyl tartrate. See Watt's *Dictionary of Chemistry*. [*Editor.*]

an old or stock solution was never used. The smaller doses were always measured by means of a tuberculin syringe graduated in hundreds of a cubic centimetre.

TABLE I

*Lethal Effects Obtained from the Administration of a 2 per cent Solution of Ammonium Antimonyl Tartrate to Guinea-pigs by Intramuscular Injection*

| Dose in gram per kilo of body weight. | Number of guinea-pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·06 | 6 | 6 | M. L. D. |
| ·055 | 4 | 3 | ... |
| ·05 | 4 | 3 | ... |
| ·045 | 6 | 4 | Maj. L. D. |
| ·035 | 6 | 2 | Maj. T. D. |
| ·03 | 6 | *Nil.* | M. T. D. |

TABLE II

*Lethal Effects Obtained from the Administration of a 2 per cent Solution of Urea Antimonyl Tartrate to Guinea-pigs by Intramuscular Injection*

| Dose in gram per kilo of body weight. | Number of guinea-pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·055 | 4 | 4 | M. L. D. |
| ·05 | 2 | 1 | ... |
| ·045 | 4 | 2 | ... |
| ·04 | 6 | 3 | ... |
| ·035 | 5 | 2 | ... |
| 03 | 3 | 1 | Maj. T. D. |
| ·025 | 4 | *Nil.* | M. T. D. |
| ·02 | 1 | *Nil.* | ... |

## Table III

*Lethal Effects Obtained from the Administration of a 2 per cent Solution of Potassium Antimonyl Tartrate to Guinea-pigs by Intramuscular Injection*

| Dose in gram per kilo of body weight. | Number of guinea-pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·055 | 4 | 4 | M. L. D. |
| ·05 | 3 | 2 | Maj L. D. |
| ·045 | 4 | 2 | ... |
| ·04 | 8 | 5 | ... |
| 035 | 6 | 3 | ... |
| ·025 | 6 | 3 | ... |
| ·02 | 6 | 2 | Maj. T. D. |
| ·015 | 3 | *Nil.* | M. T. D. |

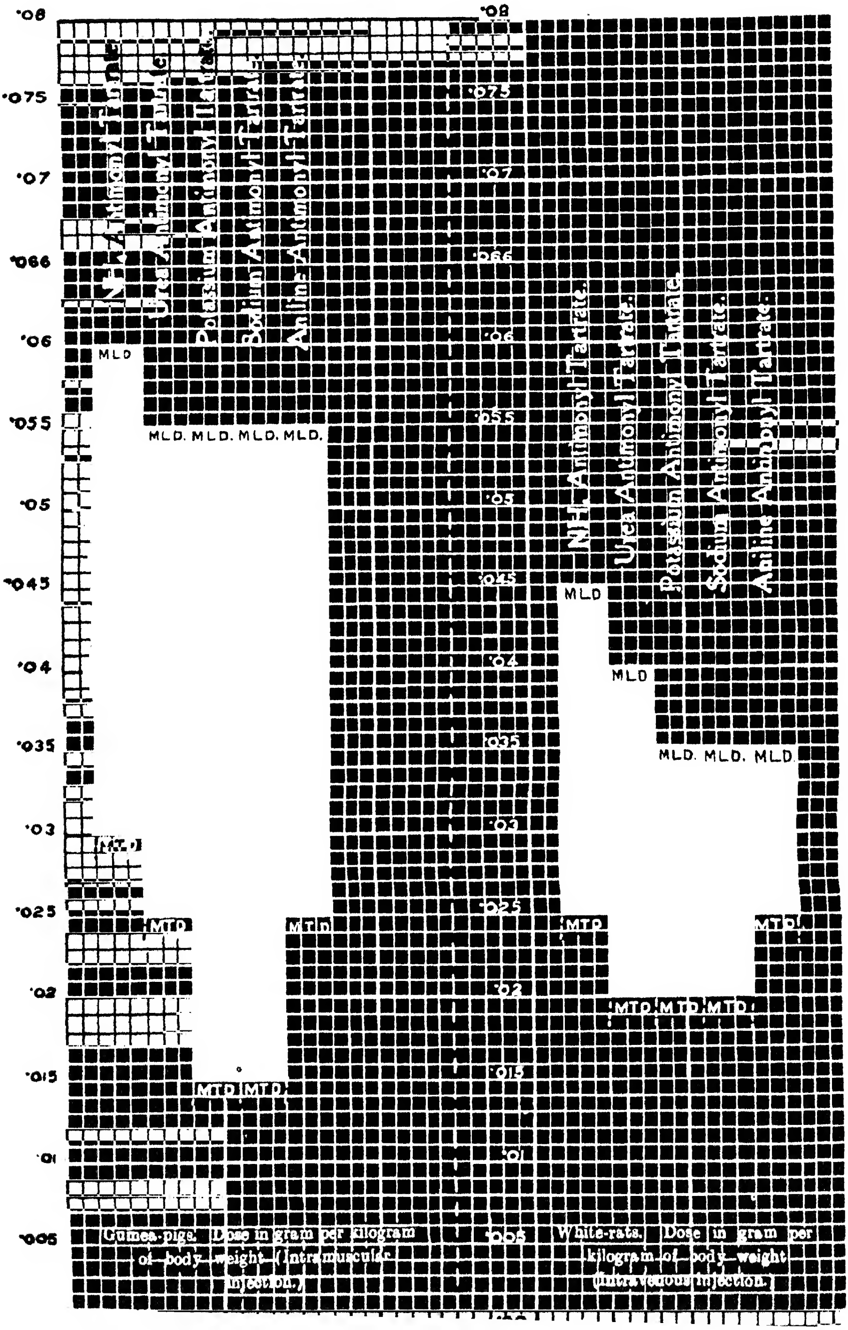
## Table IV

*Lethal Effects Obtained from the Administration of a 2 per cent Solution of Sodium Antimonyl Tartrate to Guinea-pigs by Intramuscular Injection*

| Dose in gram per kilo of body weight. | Number of guinea-pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·055 | 4 | 4 | M. L. D. |
| 05 | 3 | 2 | Maj. L. D. |
| ·045 | 4 | 2 | ... |
| ·04 | 7 | 5 | ... |
| ·035 | 6 | 3 | ... |
| ·025 | 4 | 2 | ... |
| ·02 | 5 | 1 | ... |
| ·015 | 3 | *Nil.* | M. T D. |

[Reprinted from the Indian Journal of Medical Research, Vol. X, No. 2, October, 1922]

PLATE XXIII



## TABLE V

*Lethal Effects Obtained from the Administration of a 2 per cent Solution of Aniline Antimonyl Tartrate to Guinea-pigs by Intramuscular Injection*

| Dose in gram per kilo of body weight. | Number of guinea-pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·055 | 4 | 4 | M. L. D. |
| ·05 | 4 | 3 | .. |
| ·045 | 6 | 4 | Maj. L. D. |
| ·04 | 5 | 3 | ... |
| ·03 | 4 | 2 | .. |
| 025 | 7 | *Nil.* | M. T. D. |

Represented graphically, the values obtained for the minimum lethal doses and the maximum tolerated doses of the various antimonyl tartrates for guinea-pigs will form a curve shown in the accompanying diagram (Plate XXIII).

### THE INFLUENCE OF THE BASE OF AN ANTIMONYL TARTRATE UPON ITS TOXICITY

The toxicity of a drug when administered to the same species of animals as determined from its minimum lethal dose is *inversely proportional* to its minimum lethal dose. From this the toxicity of the antimonyl tartrates and of their antimony content can be expressed as follows :—

GUINEA-PIGS

A. (1) Toxicity of ammonium antimonyl tartrate ... $= \frac{K}{\cdot 06}$

(2) Do. of urea antimonyl tartrate ... $= \frac{K}{\cdot 055}$

(3) Do. of potassium antimonyl tartrate ... $= \frac{K}{\cdot 055}$

(4) Toxicity of sodium antimonyl tartrate ... $= \frac{K}{\cdot 055}$

(5) Do. of aniline antimonyl tartrate ... $\frac{K}{\cdot 055}$.

B. (1) Toxicity of the antimony content of ammonium antimonyl tartrate

$$= \frac{K}{\cdot 06 \times 38 \cdot 1}$$

(2) Do. do. of urea antimonyl tartrate

$$= \frac{K}{\cdot 055 \times 37 \cdot 55}$$

(3) Do. do. of potassium antimonyl tartrate

$$= \frac{K}{\cdot 055 \times 36 \cdot 1}$$

(4) Do. do. of sodium antimonyl tartrate

$$= \frac{K}{\cdot 055 \times 34 \cdot 1}$$

(5) Do. do. of aniline antimonyl tartrate

$$= \frac{K}{\cdot 055 \times 31 \cdot 75}.$$

If T $(NH_4)$, T(Urea), T(K), T(Na) and T(Aniline) represent the toxicity of the above tartrates respectively, we then have :—

$$\frac{T(NH_4)}{T(Urea)} = \frac{T(NH_4)}{T(K)} = \frac{T(NH_4)}{T(Na)} = \frac{T(NH_4)}{T(Aniline)} = \frac{55}{60} \text{ or } \frac{11}{12}.$$

If T. Sb $(NH_4)$, T. Sb (Urea), T.Sb (K), T. Sb (Aniline) and T. Sb (Na) represent the toxicity of the antimony content of the above tartrates we have :—

$$\frac{T.Sb(NH_4)}{T(Urea)} = \frac{41}{46} \qquad \frac{T.Sb(NH_4)}{T.Sb(K)} = \frac{40}{46} \qquad \frac{T.Sb(NH_4)}{T.Sb(Na)} = \frac{38}{46}$$

$$\frac{T.Sb(NH_4)}{T.Sb(Aniline)} = \frac{35}{46}.$$

Therefore in the case of the guinea-pigs, ammonium antimonyl tartrate is the least toxic, then comes the urea salt, then the sodium and potassium salts which are equally toxic and then the aniline salt.

The maximum tolerating capacity of the same species of animals for a drug is *directly proportional* to its maximum tolerated dose.

We thus have :—

(1) Maximum tolerating capacity of guinea-pigs treated with ammonium antimonyl tartrate $=K' \times \cdot 03$

(2) Do. do. do. Urea antimonyl tartrate $=K' \times \cdot 025$

(3) Do. do. do. Potassium antimonyl tartrate $=K' \times \cdot 015$

(4) Do. do. do. Sodium antimonyl tartrate $=K' \times \cdot 015$

(5) Do. do. do. Aniline antimonyl tartrate $=K' \times \cdot 025$.

From this we conclude that of all the antimonyl tartrates used in the case of the guinea-pigs, their maximum tolerating capacity is with ammonium antimonyl tartrate.

## Experiments on White Rats

### *Method of Administration and Measurement of Doses*

In the case of white rats, the drugs were administered intravenously, the injection being given into one of the prominent veins of the tail. The strength of the solution was one per cent in distilled water. Whenever the injection was given, the time taken in injecting a given volume of the solution was always the same, being at the rate of ½ c.c. per minute. The injections were given by means of a tuberculin syringe and the solutions were always freshly made.

## TABLE VI

*Lethal Effects Obtained from the Administration of a 1 per cent Solution of Ammonium Antimonyl Tartrate to White Rats by Intravenous Injection*

| Dose per kilo. | Number of rats used. | Number died. | Remarks. |
|---|---|---|---|
| ·045 grm. | 4 | 4 | M. L. D. |
| ·04 ,, | 6 | 4 | Maj. L. D. |
| ·035 ,, | 6 | 4 | ... |
| ·03 ,, | 8 | 2 | ... |
| ·025 ,, | 4 | *Nil.* | M. T. D. |

## TABLE VII

*Lethal Effects Obtained from the Administration of a 1 per cent Solution of Urea Antimonyl Tartrate to White Rats by Intravenous Injection*

| Dose per kilo. | Number of rats used. | Number died. | Remarks. |
|---|---|---|---|
| ·04 grm. | 5 | 5 | M. L. D. |
| ·035 ,, | 7 | 5 | Maj. L. D. |
| ·03 ,, | 2 | 1 | ... |
| ·025 ,, | 3 | 1 | Maj. T. D. |
| ·02 ,, | 4 | *Nil.* | M. T. D. |

## TABLE VIII

*Lethal Effects Obtained from the Administration of a 1 per cent Solution of Potassium Antimonyl Tartrate to White Rats by Intravenous Injection*

| Dose per kilo. | Number of rats used | Number died. | Remarks. |
|---|---|---|---|
| ·04 grm. | 7 | 7 | ... |
| ·035 ,, | 7 | 7 | M. L. D. |
| ·03 ,, | 7 | 4 | ... |
| ·025 ,, | 5 | 3 | ... |
| ·02 ,, | 8 | *Nil.* | M. T. D. |

## TABLE IX

*Lethal Effects Obtained from the Administration of a 1 per cent Solution of Sodium Antimonyl Tartrate to White Rats by Intravenous Injection*

| Dose per kilo. | Number of rats used. | Number died. | Remarks. |
|---|---|---|---|
| ·04 grm. | 3 | 3 | ... |
| ·035 ,, | 10 | 10 | M. L. D |
| ·03 ,, | 6 | 4 | Maj. L. D. |
| ·025 ,, | 7 | 4 | ... |
| ·02 ,, | 6 | *Nil.* | M. T. D. |

## TABLE X

*Lethal Effects Obtained from the Administration of a 1 per cent Solution of Aniline Antimonyl Tartrate to White Rats by Intravenous Injection*

| Dose per kilo. | Number of rats used. | Number died. | Remarks. |
|---|---|---|---|
| ·04 grm. | 5 | 5 | ... |
| ·035 ,, | 6 | 4 | M. L. D. |
| ·03 ,, | 4 | 2 | ... |
| ·025 ,, | 5 | *Nil.* | M. T. D. |

Represented graphically the values obtained for the minimum lethal doses and the maximum tolerated doses of the various antimonyl tartrates for rats will form a curve shown in the accompanying diagram (Plate XXIII).

It will be seen from the above tables that the toxic range (T. R.) is not so great in the case of white rats as in the case of guinea-pigs and the difference in the toxicity of the various antimonyl tartrates is more marked in the case of white rats.

## SOME ARYL PENTAVALENT ANTIMONY COMPOUNDS—*p*-AMINO-PHENYL STIBINIC ACID AND SOME OF ITS DERIVATIVES—THEIR TOXICITY

Before proceeding to the study of the aromatic antimonial compounds dealt with in the present paper, I give here a brief summary of the principles of chemotherapy which have been followed in preparing them.

(1) Phenyl stibinic acid should be more toxic than *p*-amino-phenyl stibinic acid, just as phenyl arsenic acid is more poisonous than *p*-amino-phenyl arsenic acid.

(2) The sodium salt of *p*-amino-phenyl stibinic acid is the antimony analogue of atoxyl which is of marked therapeutic value in protozoal diseases. I have observed that *the urea salt is more stable and less toxic* than the sodium salt.

(3) With the idea of reducing the toxicity of *p*-amino-phenyl stibinic acid and its salts, acyl substitution compounds may be prepared by the introduction of various acidic radicles into the amino group of *p*-amino-phenyl stibinic acid to form secondary amines. Those that have been prepared are described as follows :—

(*a*) Acetyl-*p*-amino-phenyl stibinic acid and its sodium salt. The latter is identical with "Stibenyl" of Allen and Hanbury.

(*b*) Benzene-sulphonyl-*p*-amino-phenyl stibinic acid and its sodium salt. This latter is allied to Hectine of Mouneyrat.

(c) N-phenyl-glycine-amide-*p*-stibinic acid and its sodium salt. The above-mentioned acid is allied to N-phenyl-glycine-amide-*p*-arsenic acid of Jacobs and Heidelberger which has been found by Pearce and Brown to have low toxicity but marked therapeutic properties in experimental trypanosomiasis. (*Journal of Experimental Medicine*, 1919.)

(*d*) The urethano-derivative of *p*-amino-phenyl stibinic acid has been found useful in fowl spirillosis. This compound may be described as carbethoxy-*p*-amino-phenyl stibinic acid.

(4) Allyl-thio-carbamino *p*-stibanilic acid allied to allyl thiocarbamino-*p*-arsanilic acid has been prepared with the idea of having the therapeutic action of allyl and stibinic compounds without the toxic character of the latter.

(5) To reduce the toxicity of the compound phenol-*p*-stibinic acid, carboxy-methylene group may be introduced into this compound to replace the H of the OH present in the para-position of the phenolic compound giving rise to carboxy-methylene-oxyphenyl-4-stibinic acid. The corresponding arsenic compound possesses such trypanocidal power that it can cure animals infected with highly resistant strains of trypanosomes.

(6) That the introduction of acidic groups into the molecule of a compound may markedly diminish the therapeutic value of a drug has been taken into consideration.

(7) When an antimonial compound has to be used for therapeutic purposes,

$$\frac{C}{T} \quad \textit{i.e.,} \quad \frac{\text{Curative dose (Dose sufficient to kill all parasites)}}{\text{Toxic dose (Maximum dose which patient can tolerate)}}$$

should be satisfactory, which, according to Ehrlich, is $\frac{1}{3}$ or less.

It will be seen, later, that (7) can only be determined indirectly in the case of kala-azar. In this paper the term *effective dose* of a drug will be used to denote *a dose per day* by which the best effect appears to be obtained in the treatment of kala-azar, when that dose is given for a sufficient length of time. It is impossible to produce *therapia sterilisans magna* in kala-azar with a single dose of any antimonial preparation known up to the present time.

We have now to investigate how far some of the above principles of chemotherapy which are based on theoretical considerations, are borne out by actual experiments.

The starting material in the preparation of the new aromatic antimonial compounds dealt with in the present paper is acetyl-*p*-aminophenyl stibinic acid. The sodium salt of this compound is sometimes known as stib-acetin. Kala-azar and other forms of leishmaniasis have been successfully treated by its administration (G. Caronia, 1916, Pediatria. Also Kharina-Marinucii). More recently, the same compound was used by Manson-Bahr in the treatment of kala-azar under the name of "Stibenyl." (*Lancet*, Vol. II, 1920.)

The successful use of this compound in the treatment of leishmaniasis naturally leads one to attempt to prepare derivatives of this compound allied to those of *p*-arsanilic acid. The present paper contains a description of some new pentavalent antimony analogues of such derivatives of *p*-arsanilic as have been found to be of definite therapeutic value.

Acetyl-*p*-amino-phenyl-stibinic acid, $CH_3 CO.NH.C_6H_4 SbO.(OH)_2$ was prepared in my laboratory by the action of sodium antimonite upon diazo-solution in a way somewhat analogous to Bart's reaction. By diluting the sodium antimonite solution it was found that the yield was greater than that obtained by following the method described in Morgan's work on *Organic Compounds of Arsenic and Antimony*, which is the method of Von Heyden. By the latter method, the preparation is difficult and the yields are low (Percy May). The percentage of antimony in $C_8H_9O_4$ N Sb Na, the sodium salt of the above acid is 36·8. By actual calculation it was found to be 36·1.

This compound yields on hydrolysis 4-amino-phenyl-stibinic acid (Von Heyden D. R. P., 270, 488). The sodium salt of this acid is the antimony analogue of atoxyl and has been described in the German patent (Von Heyden D.R.P., 254, 421).

For the sake of simplicity I have called this sodium salt $NH_2.C_6H_4\,SbO{<}^{OH}_{ONa}$ *stibamine*, from its analogy to the corresponding salt of arsenic which is also known as arsamine (*Journal of Tropical Medicine and Hygiene*, August 15, 1921). Stibamine, as prepared in my laboratory, is an amorphous powder fairly soluble in water. Its solution is neutral. The presence of alkali or acid in its solution helps its decomposition. The solution should be freshly prepared before use.

*Composition* :—

Calculated for $C_6H_7O_3\,NSb\,Na$, Sb = 42·25 %, N = 4·93 %.

Found Sb = 42·10 %, N = 4·88 %.

(1) *Urea-stibamine*

$CO\,(NH_2)_2.\ H_2\,N\,C_6\,H_4\,SbO.\ (OH)_2$

This is carbamide salt of *p*-amino-phenyl-stibinic acid. [This compound was prepared with the idea that the urea compound could be used intramuscularly possessing anæsthetic properties as quinine urea.—*Editor*.]

## Experimental

2·3 grams of *p*-amino-phenyl-stibinic acid, suspended in water, are treated with solid urea until a clear solution is obtained on slight heating. The solution is then concentrated on the water bath. To the concentrated solution absolute alcohol is added in excess, when a precipitate forms. The mixture is heated for a few minutes to dissolve any excess of urea. The precipitate is then filtered and thoroughly washed with absolute alcohol to dissolve the last traces of uncombined urea. It is then dried on porous plate. The yield is about 1·5 grams. I propose to call this compound *urea-stibamine*.

The salt is fairly soluble in water and is amorphous.

*Composition* :—

Calculated for $C_7 H_{12} O_4 N_3 Sb$, Sb = 37·26%, N = 13·04%.

Found Sb = 36·95%, N = 12·52%.

The toxic and therapeutic properties of this compound will be described in the present paper.

(2) *Benzene-sulphonyl-p-amino-phenyl-stibinic acid*

$$C_6 H_5 . SO_2 . NH . C_6 H_4 Sb O_3 H_2$$

The sodium salt of this compound is the antimony analogue of Hectine of Mouneyrat, which is sodium-benzene-sulphonyl-*p*-amino-phenyl arsinate. This latter compound is of marked reputed value in spirochæte infection.

## EXPERIMENTAL

### *Benzene-sulphonyl-p-amino-phenyl-stibinic acid*

·5 gram of stibamine is dissolved in 2 c.c. of $\frac{N}{1}$ sodium hydroxide and treated with ·5 gram of benzene-sulphonyl chloride. The mixture is warmed on water bath at 60°C and shaken from time to time. The alkali is replenished as soon as it is found to be exhausted. After an hour and a half, the reaction is found to be complete. The solution is filtered and conc. HCl is added to it drop by drop until it is distinctly acid. The sulphonyl compound is precipitated and is then filtered. For purification, the precipitate is suspended in water and carefully dissolved in $\frac{N}{1}$ sodium hydroxide and again precipitated by hydrochloric acid. The process is repeated three times and the precipitate is filtered and carefully washed with water and dried on a porous plate. The yield is ·33 gram.

The sodium salt is a fairly stable compound and is freely soluble in water. It has not yet been obtained in a crystalline form. I propose to call it *stib-hectine.*

*Composition* :—

Calculated for $C_{12}H_{12}O_5NS.Sb$, Sb = 29·85%, N = 3·5%.
Found Sb = 29·3%, N = 4·06%.

### (3) *Urethane Derivative of p-amino-phenyl-stibinic acid (carbethoxy-p-amino-phenyl-stibinic acid)*

#### Experimental

2·9 grams of *p*-amino-phenyl-stibinate of sodium and ·6 gram of 35 per cent. caustic soda solution are dissolved in 10 c.c. of water. The mixture is treated with 1·3 grams of ethyl chlorocarbonate and 1·2 grams of 35 per cent. caustic soda solution and stirred. After about half an hour the mixture is filtered. From the filtrate urethano-derivative is precipitated with dilute hydrochloric acid. The precipitate is purified by dissolving it in caustic soda solution and then precipitating again with dilute hydrochloric acid.

*Composition* : —

Calculated for $C_9H_{12}O_5N.Sb$, Sb = 35·92%, N = 4·19%.
Found Sb = 35·74%, N = 3·96%.

### (4) *Carboxy-methylene-oxyphenyl-4-stibinic acid*

$$CO\,OH.\ CH_2.\ O.\ C_6H_4.\ Sb\,O_3\,H_2$$

#### Experimental

It is prepared by adding an alkaline solution of mono-chloracetic acid to the solution of sodium-phenol-*p*-stibinate.

Preparation of *p*-hydroxy-phenyl-stibinic acid.—The diazo solution obtained from a mixture of 2·2 grams of *p*-amino-phenol, 3 grams of sulphuric acid in 20 c.c. of

water, and 1·4 grams of sodium nitrite is added with stirring, to a sodium antimonite solution. The latter is obtained by mixing a solution of antimony trichloride, prepared by dissolving 2·88 grams of antimony trioxide in 12 c.c. of hydrochloric acid (D=1·123) and an aqueous solution of sodium hydroxide (12 grams) in 120 c.c. water. When the decomposition is complete, the excess of sodium hydroxide is almost neutralised with dilute sulphuric acid. The mixture is saturated with carbon dioxide and filtered repeatedly to remove any traces of antimony trioxide. *p*-hydroxy-phenyl-stibinate of sodium is then precipitated by saturating the solution with sodium chloride. *p*-hydroxy-phenyllstibinic acid is precipitated from the solution of the latter in water by dilute sulphuric acid. It is then filtered and dried on porous plate.

Preparation of carboxy-methylene-oxyphenyl-4-stibinic acid—It is prepared by adding successively monochloracetic acid (1·88 grams) in 3 c.c. of water and 4 grams of 35 per cent caustic soda solution to the solution of 2·63 grams of *p*-hydroxy-phenyl-stibinic acid and ·4 gram of caustic soda in 5 c.c. of water. The mixture is heated at 60°C, for about three hours. When cooled, the mixture is carefully acidified with hydrochloric acid. The precipitated acid is purified by dissolving in sodium hydroxide solution and precipitating again with dilute hydrochloric acid.

(5) *N-phenyl-glycine-amide-p-stibinic acid*

$$NH_2.OC.\ H_2\,C.\ NH.\ C_6\,H_4\ SbO\ (OH)_2$$

I have tried to investigate whether amino-phenyl-stibinic acid possesses the property of giving rise to compounds of the following type RHN. CO. $CH_2$ NH.$C_6H_4$ SbO $(OH)_2$ which are similar in constitution to those prepared by Jacobs and Heidelberger from *p*-arsanilic acid (*Journal of American Chemical Society*, 1919). Of these glycine compounds of antimony, I have prepared N-phenyl-

glycine-amide of stibinic acid which is allied to N-phenyl-glycine-amide of arsenic acid of the above authors. This latter compound has given very remarkable results in the treatment of experimental trypanosomiasis and spirochæte infection in the hands of Pearce and Brown (*Journal of Experimental Medicine*, 1919). It is therefore expected that the corresponding antimony compound prepared in my laboratory should exhibit similar results in the treatment of leishmaniasis. Its toxicity and therapeutic properties have not yet been studied by me.

## EXPERIMENTAL

### *N-(phenyl-4-stibinic acid)-glycine-amide or N-phenyl-glycine-amide-p-stibinic acid*

·8 gram of stibamine is dissolved in 4 c.c. of $\frac{N}{1}$ sodium hydroxide solution. After adding ·74 gram of chloracetamide the mixture is warmed on water bath under a reflux condenser for about two hours. During warming a reddish-brown precipitate is gradually formed and settles at the bottom, the flask being shaken from time to time. After the operation the crude product is allowed to cool. ·14 c.c. of concentrated hydrochloric acid is added to the cold mixture to hold any unchanged stibamine in solution. During this treatment the portion of the amido-glycine compound, which was retained in solution by the alkali, is precipitated. The substance is then filtered off and carefully washed with cold water. For purification it is suspended in sufficient water to form a thin paste and carefully treated by stirring with sodium hydroxide solution until the acid is dissolved. It is filtered from the undissolved product, and is then treated with a little excess of dilute acetic acid, whereupon the substance separates as a white precipitate. After filtering and washing thoroughly it is quickly dried on a porous plate and kept in a sealed tube. The yield is ·2 gram. The acid is purified by its repeated solution in alkali and precipitation by acetic acid.

*Sodium Salt.*—The pure acid is suspended in enough water to form a thick paste and carefully treated with 25 per cent sodium hydroxide solution, until completely dissolved and the solution reacts neutral to litmus. Two volumes of alcohol are then added, the pure sodium salt quickly separating as a white powder. After filtering and washing with 85 per cent alcohol it is quickly dried on a porous plate.

The acid is sparingly soluble in cold water. It dissolves more easily in hot water. The sodium salt is freely soluble in water. It has not yet been obtained in a crystalline form and is less soluble in water than the corresponding arsenic compound. I propose to call this compound *stib-glycine-amide.*

*Composition* :—

Calculated for $C_8 H_{10} O_4 N_2$ Sb Na, Sb=35·19%, N=8·2%.

Found Sb=35·41%, N=7·9%.

(6) *Allyl-thio-carbamino-p-amino-phenyl-stibinic acid*

$$C_3 H_5 NH.\ CS.\ NH.\ C_6 H_4 SbO\ (OH)_2$$

The above compound is prepared by treating stib-amine with allylthiocarbamide in methyl alcohol.

## Experimental

·2 gram stibamine is dissolved in 3 c.c. of methyl alcohol and to the mixture ·08 grm. oleum sinapis (containing 90 per cent allylthiocarbamide) is added. The mixture is kept at ordinary temperature for 24 hours, and then filtered. The filtrate is diluted with a little water and treated with a few drops of concentrated hydrochloric acid, which precipitates the allylthiocarbamino derivative. The crude product is filtered and washed with water and dried in a desiccator. The dried substance is finally washed with ether, to free it from oil. The compound obtained is a yellowish white

amorphous powder, soluble in sodium hydroxide solution, but not soluble in sodium carbonate The yield is ·2 gram.

*Composition* :—

Calculated for $C_{10}\ O_3\ H_{13}\ N_2$ S. Sb, Sb = 33.2%, N = 7·7%.

Found Sb = 33·4%, N = 7·8%.

In the present paper, the toxicity and therapeutic properties of urea-stibamine will be described. The toxicity of some of the other aryl antimonial compounds dealt with in the present paper will also be described here.

*Toxicity Experiments with Phenyl Stibinate of Sodium Stibamine, Urea Stibamine, etc.*

*Method of administration.*—The drugs were administered into guinea-pigs intramuscularly, the injections being given in the outer part of the thigh. The strength of the solution was 2 per cent in distilled water. In all these experiments, each time the solution was freshly prepared and an old solution was never used.

TABLE XI

*Lethal Effects Obtained from the Administration of a 2 per cent Solution of Phenyl Stibinate of Sodium to Guinea-pigs by Intramuscular Injection*

| Dose per kilo. | Number of guinea-pigs used | Number died. | Remarks. |
|---|---|---|---|
| ·2 grm. | 2 | 2 | .. |
| ·1 ,, | 2 | 2 | . |
| ·05 ,, | 3 | 3 | ... |
| 025 ,, | 4 | *Nil.* | ... |

## TABLE XII

*Lethal Effects Produced from the Administration of a 2 per cent Solution of acetyl-p-amino-phenyl-stibinate of Sodium to Guinea-pigs by Intramuscular Injection (Stibenyl)*

| Dose per kilo. | Number of guinea-pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·7 grm. | 3 | 3 | M. L. D. |
| ·6 ,, | 5 | 3 | ... |
| ·5 ,, | 4 | 2 | ... |
| ·45 ,, | 4 | 2 | ... |
| ·4 ,, | 6 | 1 | ... |
| ·35 ,, | 2 | *Nil.* | M. T. D. |

## TABLE XIII

*Lethal Effects Produced from the Administration of a 2 per cent Solution of Urea Stibamine to Guinea-pigs by Intramuscular Injection*

| Dose per kilo. | Number of guinea-pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·7 grm. | 4 | 4 | M. L. D. |
| ·65 ,, | 3 | 2 | Maj. L. D. |
| ·6 ,, | 4 | 2 | ... |
| ·5 ,, | 2 | 1 | ... |
| ·45 ,, | 4 | 1 | ... |
| ·4 ,, | 4 | 1 | ... |
| ·35 ,, | 4 | *Nil.* | M. T. D. |

## TABLE XIV

*Lethal Effects Produced from the Administration of a 2 per cent Solution of Stibamine to Guinea-pigs by Intramuscular Injection*

| Dose per kilo. | Number of guinea pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·5 grm. | 4 | 4 | M. L. D. |
| ·45 ,, | 4 | 3 | ... |
| ·4 ,, | 4 | 3 | ... |
| ·35 ,, | 4 | 2 | .. |
| ·3 ,, | 8 | 2 | ... |
| ·2 ,, | 6 | *Nil.* | M. T. D. |

## TABLE XV

*Lethal Effects Produced from the Administration of 2 per cent Solution of Stib-hectine to Guinea-pigs by Intramuscular Injection (Compound made in my Laboratory)*

| Dose per kilo. | Number of guinea pigs used. | Number died. | Remarks. |
|---|---|---|---|
| ·6 grm. | 4 | 4 | ... |
| ·5 ,, | 4 | 4 | M. L. D. |
| ·4 ,, | 3 | 2 | ... |
| ·3 ,, | 3 | 2 | .. |
| ·2 ,, | 2 | 1 | ... |

TABLE XVI

*Lethal Effects Produced from the Administration of a 2 per cent Solution of Stib-hectine to Guinea-pigs by Intramuscular Injection (Compound supplied by Chemisch. Fabrik. von Heyden)*

| Dose per kilo. | Number of guinea-pigs used. | Number died | Remarks. |
|---|---|---|---|
| ·5 grm. | 3 | 3 | ... |
| ·4 ,, | 5 | 5 | M. L. D. |
| ·3 ,, | 4 | 3 | ... |
| ·25 ,, | | 1 | ... |

*Symptoms of poisoning after intramuscular injection of aryl antimonials into guinea-pigs.*—These symptoms are, generally speaking, similar to those following toxic doses of the antimonyl tartrates. The pathological changes in the organs after toxic doses of the aryl antimonials are also similar to those obtained after administration of toxic doses of antimonyl tartrates. In one case in which the optic nerve was examined, I did not find such degenerative changes in the optic nerve as have been observed to have followed the use of the aryl arsonates.

Having determined the toxicity of stibamine and urea stibamine, I give a summary of their physical and chemical properties.

## PHYSICAL AND CHEMICAL PROPERTIES OF STIBAMINE AND UREA-STIBAMINE

*Properties of stibamine :*—

(1) Stibamine is a brown amorphous powder soluble in water, the solution being of a reddish-yellow colour.

(2) The solution of stibamine is easily decomposed, giving rise to a precipitate containing antimony, in the presence of an acid or alkali.

(3) The filtrate after separation of the above precipitate also contains antimony.

*Properties of urea stibamine* :—

(1) It is a brown amorphous powder like stibamine and is soluble in water giving rise to a reddish solution. It is insoluble in alcohol.

(2) Unlike stibamine, its solution is not so easily decomposed by boiling for a few minutes. Its solution can be sterilized by boiling.

(3) It is more stable than stibamine when kept in solution.

(4) It is an additive compound of urea and liberates $N_2$ when treated with a solution of sodium hypobromite.

*The therapeutic value of ammonium antimonyl tartrate and urea stibamine* :—

Having proved that ammonium antimonyl tartrate is the least toxic of the five antimonyl tartrates investigated in the present paper, I now pass on to describe its effects when administered to man for therapeutic purposes. Only a few clinical cases will be described here. It is, however, beyond the scope of the present paper to give the comparative value of the various antimonyl tartrates in the treatment of kala-azar.

## Treatment of Kala-azar with Intravenous Injection of Ammonium Antimonyl Tartrate

(1) Patient K, æt. 30, was admitted into hospital suffering from kala-azar. The spleen extended $5\frac{1}{2}''$ below the costal margin. On spleen puncture L. D. bodies were found. At the time of admission the body weight was 5 stone. Patient was treated with intravenous injections of ammonium antimonyl tartrate twice a week, the doses being increased from 2 c.c. to 8 c.c. of a 2 per cent solution. Altogether

16 injections were given. As a result of the treatment, the fever of the patient completely stopped, the spleen could not be felt below the costal margin and on spleen puncture no L. D. bodies could be found after the 16th injection. Patient increased one stone in weight during the treatment.

*Result of Blood Examination* :—

(1) R.B.C.—2,300,000, W.B.C.—2,000, Hb—32 per cent on 15-6-1921 before treatment.

(2) R.B.C.—4,300,000, W.B.C.—7,000, Hb—55 per cent on 12-9-1921 after treatment.

(3) Patient N, æt. 16, was admitted into hospital suffering from kala-azar. The spleen extended 4″ below the costal margin. On spleen puncture many L.D. bodies were found. At the time of admission the body weight was 3 stone. Patient was treated with intravenous injections of ammonium antimonyl tartrate twice a week, the doses being increased from 1 c.c. to 6 c.c. of a 2 per cent solution. Altogether 25 injections were given. As a result of treatment, the fever of the patient completely stopped, the spleen could not be felt below the costal arch after the 20th injection, and at the time of discharge no L.D. bodies could be found on spleen puncture. Patient increased 1 stone in weight during the treatment.

*Result of Blood Examination* :—

(1) R.B.C.—3,200,000, W.B.C.—2,600. Hb—44 per cent on 3-6-1921 before treatment.

(2) R.B.C.—4,500,000, W.B.C—7,000, Hb—60 per cent on 1-6-1921 after treatment.

(3) Patient A, æt. 21, was admitted into hospital suffering from kala-azar. The spleen extended 7″ below the costal margin. At the time of admission the body weight was 6 stone. Patient was treated with intravenous injections of ammonium antimonyl tartrate twice a week, the doses being

increased from 3 c.c. to 9 c.c. of a 2 per cent solution. Altogether 14 injections were given. As a result of treatment, the fever subsided, the spleen could just be felt below the costal margin after the 15th injection and at the time of discharge no L. D. bodies could be found on spleen puncture. Patient increased 1 stone in weight during the treatment.

*Result of blood examination* :—

(1) R.B.C.—2,900,000, W.B.C.—1,200, Hb—42 per cent on 30-6-1921 before treatment.

(2) R.B.C.—4,200,000, W.B.C.—11,400, Hb—55 per cent on 5-11-1921 after treatment.

Each of the above cases appeared to be cured. It will be seen that the highest dose given up to now was 9 c.c. of a 2 per cent solution. Symptoms of vomiting and purging were not great after these injections. A series of cases which could not bear treatment with tartar emetic, on account of severe reactions, such as high fever, vomiting and purging, are now being treated with ammonium antimonyl tartrate with less marked reactions. The intramuscular injection of the compound is painful, and may give rise to local reaction, which is not so marked as in the case of tartar emetic.

## Treatment of Kala-azar with Intravenous Injection of Urea Stibamine

In the following cases of kala-azar the effects of intravenous injection of urea stibamine are briefly recorded.

(1) Name—Manu, æt. 10 years. L. D. bodies found on spleen puncture. Dose = ·15 gram given twice a week.

*Effect of treatment* :—

| R.B.C. | W.B.C. | Hb |
|---|---|---|
| 4,200,000 | 3,200 | 50 per cent on admission. |
| 3,100,000 | 4,800 | 40 ,, after 5 injections. |
| 3,400,000 | 4,800 | 48 ,, ,, 16 ,, |
| 3,200,000 | 10,400 | 46 ,, ,, 20 ,, |

Spleen reduced from $3\frac{1}{2}''$ to $1\frac{1}{2}''$ below the costal arch. No L. D. bodies found after 20 injections. Patient free from fever for one month.

(2) Tofu, æt. 30 years. L. D. bodies found on spleen puncture before treatment.

$$\text{Dose} = \begin{cases} (1)\ 10\ \text{c.c.} - 2\ \text{injections}\ (0{\cdot}2\ \text{gram}). \\ (2)\ 12\frac{1}{2}\ \text{c.c.} - 13\ \quad ,,\quad (0{\cdot}25\ \text{gram}). \\ (3)\ 15\ \text{c.c.} - 3\ \quad ,,\quad (0{\cdot}3\ \text{gram}). \end{cases}$$

Injections given twice a week.

*Effect of treatment* :—

| R.B.C. | W.B.C. | Hb |
|---|---|---|
| 2,900,000 | 1,800 | 38 per cent on admission. |
| 2,800,000 | 4,200 | 38 ,, after 3 injections. |
| 3,200,000 | 5,200 | 42 ,, ,, 14 ,, |
| 4,700,000 | 10,400 | 52 ,, ,, 18 ,, |

Spleen reduced from $2\frac{1}{2}''$ to *nil* beneath costal arch. Body weight increased from 5 st. $2\frac{1}{2}$lb. to 6 st. 3 lb. No L. D. bodies found on spleen puncture after 18 injections. Patient free from fever for one month.

(3) Abdul, æt. 30 years. Spleen 6″ below the costal arch in the left nipple line and 2″ away from mid-line to the right side. L. D. bodies found on spleen puncture before treatment.

Dose = ·2 gram at each injection. Injections given twice a week.

*Effect of treatment* :—

| R.B.C. | W.B.C. | Hb |
|---|---|---|
| 1,900,000 | 1,800 | 28 per cent on admission. |
| 3,000,000 | 2,600 | 44 ,, after 7 injections. |
| 3,900,000 | 3,600 | 50 ,, ,, 13 ,, |

Spleen slightly felt below the costal arch and body weight increased from 6 st. 6 lb. to 7 st. 4 lb. Patient discharged at his own request. Patient free from fever for one month.

(4) Anath Bondhu, æt. 14 years. L. D. bodies found on spleen puncture before treatment.

Dose = ·2 gram twice a week.

*Effect of treatment* :—

| R.B.C. | W.B.C. | Hb |
|---|---|---|
| 2,600,000 | 1,200 | 38 per cent on admission. |
| 2,800,000 | 4,200 | 42 ,, after 5 injections. |
| 3,800,000 | 8,000 | 50 ,, ,, 12 ,, |

Spleen reduced from 6″ to almost *nil* below the costal arch. Body weight increased from 4 st. 21 lb. to 5 st. 6 lb. Patient free from fever for one month.

(5) Abdul, æt. 12 years. Spleen 4½″ below the costal arch. L. D. bodies found on spleen puncture before treatment.

Dose = ·15 gram twice a week.

*Effect of treatment* :—

| R.B C. | W.B.C. | Hb |
|---|---|---|
| 2,900,000 | 1,400 | 44 per cent on admission. |
| 3,700,000 | 2,400 | 48 ,, after 10 injections. |
| 4,200,000 | 2,400 | 50 ,, ,, 14 ,, |
| 3,900,000 | 5,200 | 42 ,, ,, 16 ,, |

Patient absconded from hospital.

(6) Mosafar, æt. 30 years. L. D. bodies found on spleen puncture before treatment.

Doses.—1st $2\frac{1}{2}$ c.c., 2nd 5 c.c., 3rd 10 c.c. and the last 6 injections $12\frac{1}{2}$ c.c. of a 2 per cent solution. Injections given twice a week.

*Effect of treatment* :—

| R.B.C. | W.B.C. | Hb |
|---|---|---|
| 2,400,000 | 1,600 | 32 per cent on admission. |
| 2,400,000 | 7,000 | 36 ,, after 9 injections. |

Patient absconded from hospital.

(7) Horoz, æt. 10 years. L. D. bodies found on spleen-puncture before treatment. Spleen extended $5\frac{1}{2}''$ below the costal arch before treatment.

Dose = ·05 to ·15 gram every alternate day.

*Effect of treatment* :—

| R.B.C. | W.B.C, | Hb |
|---|---|---|
| 2,300,000 | 2,000 | 36 per cent before treatment. |
| 3,400,000 | 3,800 | 40 ,, after 3 injections. |
| 4,200,000 | 13,800 | 46 ,, ,, 16 ,, and same after 27 injections. |

Spleen just felt below costal arch. Patient free from fever for one month.

No L. D. bodies found on spleen puncture after 20 injections.

(8) Abdul, æt. 25. L. D. bodies found on spleen puncture before treatment.

Dose = ·25 gram twice a week.

*Effect of treatment* :—

| R.B.C. | W.B.C. | Hb | |
|---|---|---|---|
| 3,000,000 | 2,400 | 40 per cent | on admission. |
| 4,300,000 | 7,000 | 50 ,, | after 7 injections. |

Spleen reduced from 7″ to almost *nil* below the costal arch. Body weight increased from 6 st. to 7 st. 4 lb.

Patient left hospital before treatment was completed.

## REMARKS

In the present paper, the toxicity of some of the antimonyl tartrates and some of new aromatic antimonials has been described.

In his observation on the Treatment of Oriental Sore, Greig has come to the following conclusions with regard to the use of tartar emetic in the treatment of the disease : "It is not desirable to exceed 12 to 13 c.c. (1 per cent solution) at one time as toxic symptoms become more marked above this limit. Hence we see that the $\frac{C}{T}$ dose ratio of antimonium tartaratum is not very satisfactory, the organo- and parasito-tropic properties are not in the correct proportion. ......The ideal drug for the destruction of *Leishmania tropica* in the tissues has still to be sought." (*Indian Journal of Medical Research*, October, 1917.) The same also holds good in the use of the drug in the treatment of kala-azar. The average minimum effective dose of tartar emetic in the case of an adult man in the treatment of kala-azar may be taken as 6 c.c. of a 2 per cent solution (=·12 gram). The average minimum effective dose of urea stibamine used for the same purpose is ·25 gram. If C and C′ denote these minimum effective doses respectively, we have $\frac{C}{C'} = \frac{\cdot 12}{\cdot 25}$ or $\frac{1}{2}$ approximately.

From the toxicity experiments described in the present paper it will be seen that the maximum tolerated doses per kilo of body weight in the case of the guinea-pig are ·015 gram of tartar emetic and ·35 gram of urea stibamine. If T and T′ represent these tolerated doses we have :—

$$\frac{T}{T'} = \frac{\cdot015}{\cdot35} \text{ or } \frac{1}{23} \text{ nearly.}$$

Though it does not necessarily follow that the minimum tolerated dose for the human being can be reckoned weight for weight by rule of three with mathematical accuracy from observations on the guinea-pig, still it is evident from the above figures that urea stibamine is a much safer antimonial for use in the treatment of kala-azar than tartar emetic. The fact also holds good in the case of the other antimonyl tartrates. The effective dose of urea stibamine in the treatment of kala-azar is $\frac{5}{7}$ths the tolerated dose for the guinea-pig, while in the case of tartar emetic, it is 8 times the tolerated dose for the same animal.

## Conclusions

(1) After the administration of a toxic dose of an antimonyl tartrate, the pathological changes are most markedly seen in the lungs, the kidneys, the liver, pituitary and adrenals. These consist chiefly of hæmorrhages into the substance of these organs and destruction of their cellular elements. Similar pathological changes are also observed after toxic doses of the aromatic antimonial compounds.

(2) Ammonium antimonyl tartrate is the least toxic of all the antimonyl tartrates used.

(3) The toxicity of the *antimony content* of an antimonyl tartrate is least marked in the case of the ammonium salt.

(4) The presence of N in the basic radicle of an antimonyl tartrate diminishes the toxicity of some of them.

(5) Ammonium antimonyl tartrate is of marked therapeutic value in the treatment of kala-azar.

(6) The low toxicity of ammonium antimonyl tartrate and its high antimony content lead to the conclusion that of all the antimonyl tartrates dealt with in the present paper, ammonium antimonyl tartrate is the best for use in the treatment of kala-azar.

(7) A series of new organic aromatic antimonials have been discovered, the preparations of which have been described in the body of the paper.

(8) The toxicity of the following aromatic antimonials has been estimated in the case of the guinea-pig : (1) Phenyl stibinic acid, (2) Acetyl-*p*-amino-phenyl stibinic acid, (3) Stibamine, (4) Urea stibamine, (5) Stib-hectine.

(9) The acetyl derivative of *p*-amino-phenyl stibinic acid is less toxic than stibamine.

(10) Urea stibamine is less toxic than stibamine.

(11) Urea stibamine has been found useful in the treatment of kala-azar.

(12) Urea stibamine is a much safer antimonial for use in the treatment of kala-azar than tartar emetic or other antimonyl tartrates.

(13) Symptoms, such as vomiting and purging, are much less marked after intravenous injection of an effective dose (=·25 gram) of urea stibamine than that of tartar emetic or sodium antimonyl tartrate (=·12 gram).

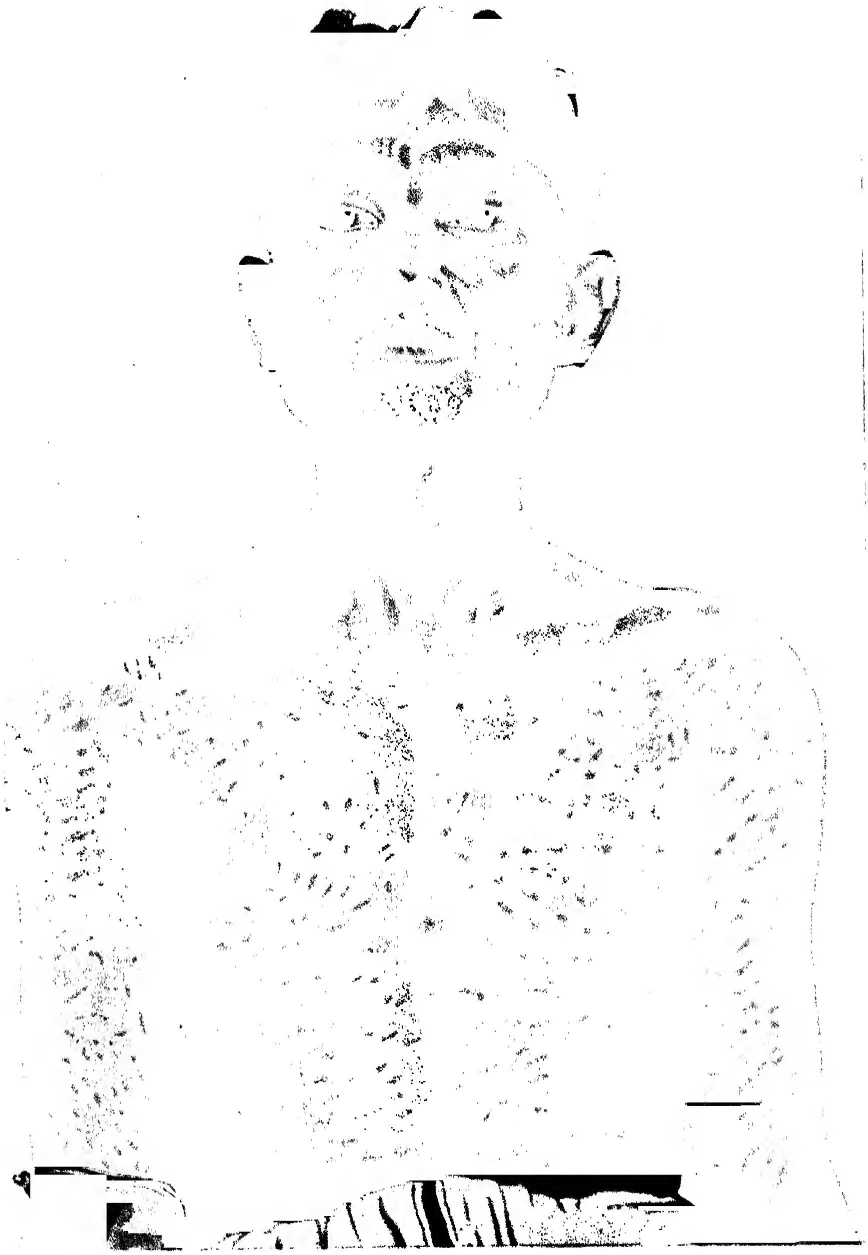
I am deeply indebted to my staff, Mr. Niharranjan Chatterjee, M.Sc., Mr. Saradacharan Chaudhury, M.Sc., Dr. Pramathanath Ghose, M.B., and Sub-Assistant Surgeon Bibhutibhushan Maity, L.S.M.F., for helping me in carrying

on my researches. My grateful thanks are also due to Mr. Parimal Sen, M.Sc., for the drawings and sections of all the organs described in this paper.

[*N.B.*—The paper of Fargher and Gray on the Chemotherapy of Antimony, which was published after my paper was sent to the Secretary, Indian Research Fund Association, last December, will be discussed in a subsequent communication.]

[Reprinted from the Indian Journal of Medical Research, Vol. X, No. 4, April, 1922]

PLATE LXXIII



[*Reprinted from the Indian Journal of Medical Research, Vol. X, No. 4, April, 1923*]

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

## PART II

### DERMAL LEISHMANOID

[Received for Publication, June 5, 1922]

Under the name of *Dermal Leishmanoid* I described, in the April, 1922 issue of the *Indian Medical Gazette*, a form of dermal leishmaniasis, which developed in a case of kala-azar cured by antimonial treatment. Since the publication of this paper, Major Knowles, I.M.S., has made a series of inoculations and cultural experiments, which are described below.

I am indebted to the Editor, *Indian Medical Gazette*, for permission to reproduce here a drawing showing the eruptions on the upper part of the patient's body (Plate LXXIII). A drawing from the scrapings from one of the papules is also appended herewith showing the presence of *Leishmania donovani*, which seem to be mostly extra-corpuscular, in the smear. A few have been found inside leucocytes and endothelial cells. (Plate LXXIV, Fig. 1.)

### INOCULATION AND CULTURAL EXPERIMENTS

A series of experiments have been performed to determine if flagellates developed from the Leishman-Donovan bodies which had apparently been modified in their virulence by a course of antimonial treatment and also to determine if they

could infect monkeys by giving rise to a local or general disease.

The following are the notes on the cultural and inoculation experiments very kindly made for me by Major Knowles, I.M.S., Protozoologist, Calcutta School of Tropical Medicine :—

(1) One of the nodules of the right arm was pricked and the serum which oozed out was inoculated into NNN medium and incubated at 22°C. Flagellated bodies were found after 12 days, and these were indistinguishable from those of *Leishmania donovani*. (Plate LXXIV, Fig. 2.)

(2) The culture from the peripheral blood of the patient and smears from the same gave negative results. Examination of the splenic blood—negative.

(3) A monkey (*M. rhesus*) was inoculated in both eyebrows by embedding bits of granulomatous nodules into pockets cut in them. After a month-and-a-half, marked granulomatous growths were observed over the sites of inoculation in both eyebrows. (See Diagram.) Also small secondary nodules were observed at the outer and inner canthuses of the eyes. One of the nodules at the original site of inoculation was incised and smears made from it—a fair number of Leishman-Donovan bodies were present, most of which were intra-corpuscular and a few extra-corpuscular and free. (Plate LXXIV, Fig. 3.)

(4) No ulceration has yet been observed over the nodules after two-and-a-half months, the raw surface left after incising one of the nodules having healed up.

(5) Blood examination and culture from the peripheral blood of the monkey were negative a month-and-a-half after inoculation.

(6) The smears from the liver of the monkey and cultures from the same organ on NNN medium gave negative results a month-and-a-half after inoculation.

Fig. 1, Plate LXXIV—scrapings from one of the papules, showing the presence of L. D. bodies which are mostly free forms and extracapsular, a few are inside leucocyte and endothelial cells. (Vide also p. 53)

Fig. 2, Plate LXXIV—scrapings showing flagellated bodies found after 12 days which were indistinguishable from those of L. D. bodies.

Fig. 3, Plate LXXIV, shows a fair number of L. D. bodies in smears from one of the incised nodules taken from the original site of inoculation made in the monkey.

Fig. 4, Plate LXXIV—section of a papule in the skin of the patient showing round-celled infiltration with fibroblasts and thinning af the epidermis.

Fig. 5 is the same as Fig. 4 showing the presence of a network of newly formed capillaries and thickening of capillary walls.

[Reprinted from the Indian Journal of Medical Research, Vol X, No. 4, April, 1923]

PLATE LXXIV

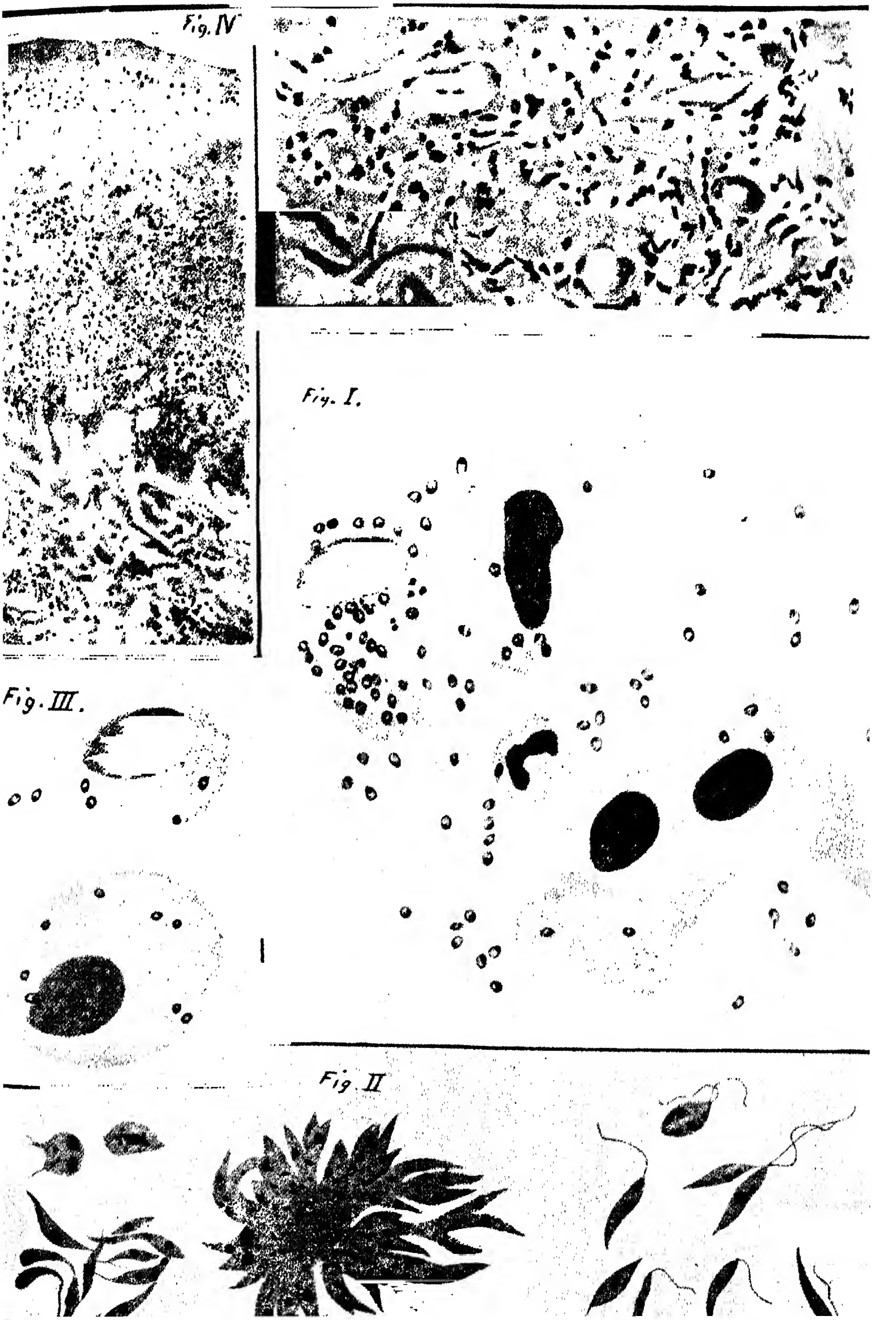


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PLATE LXXIV (b)

Photograph of a monkey, showing marked granulomatous growths at the site of inoculation in both eyebrows done a month and a half before, by embedding bits of granulomatous nodules into pockets cut in them, with secondary nodules at the outer and inner canthuses of the eyes.

## Histo-pathology of the Granuloma

The normal structure of the chorium is replaced by granulation tissue consisting mainly of large cells which are arranged in columns and between them young fibroblasts and fine capillaries of the granulation tissue are seen. Here and there in the large capillaries the endothelium is hypertrophied and the wall is thickened. In some places there is a very marked thickening of the wall of the capillaries almost leading to their obliteration. On the wall of the thickened capillaries and in their endothelial lining are seen Leishman-Donovan bodies. The skin over the papillomatous nodules is moderately pigmented and stratum cornium is very thin. The papillæ of the chorium are much less prominent than the normal ones. There is no surface ulceration. No Leishman-Donovan bodies are seen in the epidermis. On the whole, the pathological changes in the skin are very similar to those recently described by Cornwall in a non-ulcerated oriental sore. (Plate LXXIV, Figs. 4 and 5.)

The Leishman-Donovan bodies are best seen in smears from the scrapings from the granulomatous nodules. In these smears they appear mostly as free parasites. Here and there, on careful examination, they are found also inside large mononuclear leucocytes and very rarely inside the polynuclears. Besides, they are found inside other large cells which are perhaps endothelial cells. (Plate LXXIV, Fig. 1.)

After the publication of the case the patient underwent a course of antimonial treatment alternated with intravenous injection of salvarsan and seemed to improve somewhat. But he left Calcutta before the completion of his treatment.

Leishman-Donovan bodies have been occasionally discovered in the papular eruptions and scrapings from

ulcers in the skin in cases of kala-azar (Christophers). The findings in the above case of dermal leishmanoid, however, differentiate it from such cases, as, so far as can be made out, the present case is purely a local *Leishmania* infection of the skin in a patient otherwise cured of kala-azar.

## OBSERVATIONS

(1) The granulomatous nodules of the skin contain *Leishmania donovani*, most of which are extra-cellular and some intra-cellular—the reverse of what occurs in oriental sore.

(2) The culture from the serum from the granulomatous nodules produced flagellate forms indistinguishable from those of *Leishmania donovani*.

(3) An inoculated monkey developed granulomatous nodules containing *Leishmania donovani* which are mostly intra-cellular but some are extra-cellular, just the reverse of what was found in the patient and more resembling the findings in oriental sore.

(4) The disease in the case of the monkey is still a local disease. But no ulceration has yet been observed in the nodules of the inoculated monkey.

(5) The patient's peripheral blood and splenic blood gave negative results.

(6) The pathological changes in the skin are very similar to those observed in oriental sore.

The facts, established by the cultural and inoculation experiments described in the present paper, that the disease is purely a lesion of the skin, that no *Leishmania* could be cultivated from the peripheral blood, that the examination of the patient's splenic blood was negative, and that in the successfully inoculated monkey no *Leishmania* could be cultivated from the peripheral blood and the liver, all appear to support my view that the disease was due to a modified

virus of *Leishmania donovani*. The histological changes in the skin resemble those of a non-ulcerated oriental sore, with this difference that in the present disease the *Leishmania* are mostly extra-corpuscular while in oriental sore they are mostly intra-corpuscular. The reverse condition has, however, been found in the monkey. These facts lead me to conclude that the modified virus of *Leishmania donovani* brought about by antimonial treatment resembles that of *Leishmania tropica*, the causative agent of oriental sore.

## CONCLUSIONS

The case is therefore one of cutaneous leishmaniasis due to *Leishmania donovani* and proves for the first time that these parasites can sometimes produce only skin manifestations in man, without visceral lesions. This condition has followed antimonial treatment of kala-azar.

My grateful thanks are due to Major Knowles, I.M.S., for the invaluable help he has given me in making the cultural and inoculation experiments. To my assistant, Mr. Parimal Bikas Sen, M.Sc., I am greatly indebted for the drawing of the Plates for my paper.

[Reprinted from the Indian Journal of Medical Research, Vol. XI, No. 1, July, 1923]

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

## PART III

## FURTHER OBSERVATIONS ON THE TOXICITY OF ANTIMONIAL COMPOUNDS—DELAYED ANTIMONY POISONING

[Received for Publication, December 19, 1922]

## (A)

## FURTHER OBSERVATION ON THE TOXICITY OF ANTIMONIAL COMPOUNDS

### EXPERIMENTS ON GUINEA-PIGS

The same methods of administration and measurement of doses were followed as in my previous paper. These will not therefore be described again.

### (1) *Lithium antimonyl tartrate*

Method of preparation :—

### EXPERIMENTAL

·37 grm. of lithium carbonate is slowly added to a watery solution of 1·5 grms. of tartaric acid. The mixture is heated till all the $CO_2$ is expelled. To the solution 1·44 grms. of $Sb_2O_3$ are added and the mixture gently heated till all the $Sb_2O_3$ dissolves. The solution is filtered and concentrated and to the concentrated solution is added four times its volume of absolute alcohol. Lithium antimonyl tartrate is precipitated as granular crystals.

Yield—2·4 grams.

Calculated for $LiC_4H_4O_7Sb$, Sb = 41·2 per cent.

Found Sb = 40·6 per cent.

Lethal effects obtained from the administration of a two per cent solution of lithium antimonyl tartrate into guinea-pigs by intramuscular injection :—

TABLE I

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·045 | 4 | 4 |
| ·04 | 6 | 6 |
| ·03 | 4 | 3 |
| ·02 | 4 | Nil |

$$\text{Toxicity of antimony content} = \frac{K}{\cdot04 \times 40{\cdot}6} \text{ or } \frac{K}{1{\cdot}624}.$$

(2) *Calcium antimonyl tartrate*

Method of preparation :—

EXPERIMENTAL

·56 grm. of anhydrous calcium oxide is dissolved in a watery solution of 3 grms. of tartaric acid. To the mixture 2·88 grms. of $Sb_2O_3$ are slowly added, while it is heated on the sand bath. When all the antimony trioxide has gone into solution, the solution is filtered, concentrated and allowed to crystallize. It is purified by recrystallization from water.

Yield—5 grams.

Heated at 110°C, found loss of weight = 14·8 per cent.

Calculated for $CaC_8H_8O_{14}Sb_2.6\frac{1}{2}H_2O$, weight of water of crystallization = 16·1 per cent.

Calculated for the above hydrated salt, Sb = 33·1 per cent.
Found Sb = 33·0 per cent.

Lethal effects produced from the administration of a two per cent solution of calcium antimonyl tartrate into guinea-pigs by intramuscular injection :—

TABLE II

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·055 | 2 | 2 |
| ·05 | 4 | 3 |
| ·045 | 4 | 3 |
| ·02 | 4 | 0 |

Toxicity of antimony content $= \frac{K}{\cdot 055 \times 33}$ or $\frac{K}{1\cdot 815}$.

(3) *Strontium antimonyl tartrate*

Method of preparation :—

EXPERIMENTAL

To a watery solution of 1·5 grms. of tartaric acid is slowly added ·73 grms. of strontium carbonate. The mixture is then heated till all the $CO_2$ is expelled. The solution is then heated on the sand bath and during the process 1·44 grms. of $Sb_2O_3$ are slowly added to it and the heating is continued till all the $Sb_2O_3$ dissolves. The solution is then filtered, concentrated and allowed to crystallize. It is purified by crystallization from water.

Yield—3 grams.

Calculated for $SrC_8H_8O_{14}Sb_2$, Sb = 36·66 per cent.

Found Sb = 36·6 per cent.

Lethal effects produced from the administration of a two per cent solution of strontium antimonyl tartrate into guinea-pigs by intramuscular injection :—

TABLE III

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·055 | 3 | 3 |
| ·05 | 4 | 3 |
| ·045 | 7 | 6 |
| ·04 | 2 | 1 |
| ·03 | 2 | 1 |
| ·02 | 2 | 1 |

Toxicity of antimony content $= \frac{K}{\cdot055 \times 36\cdot6}$ or $\frac{K}{2\cdot01}$ .

(4) *Ethyl antimonyl tartrate*

Method of preparation :—

Ethyl antimonyl tartrate was prepared in solution by Prof. Collie by heating freshly precipitated antimony trioxide with acid ethyl tartrate, to about 150°C. in a sealed tube. [*Pro. Roy. Sco.*, 82, B (1910)—252.) The latter can be prepared after the method described by Guerin-Varry—A, 1837, 22, 238 (*vide* Sudborough's *Practical Organic Chemistry*). Another method is that of J. Bongault [*J. Pharm. Chim.*, 1906 (VI), 23. 465-69.]

The best method of preparing ethyl antimonyl tartrate is by the action of ethyl iodide on silver antimonyl tartrate in presence of absolute alcohol.

EXPERIMENTAL

Silver antimonyl tartrate is prepared by adding a solution of silver nitrate (2·6 grams) to a concentrated solution of

tartar emetic (5 grams). This yields white granular crystals of silver antimonyl tartrate. The precipitated silver salt is filtered, washed with cold water and then dried in air.

4·1 grms. of silver antimonyl tartrate dried at 105°C. are mixed in a conical flask, with about 20 c.c. of absolute alcohol and excess of ethyl iodide. The mixture is gently heated on the water bath for about five hours under reflux. After the completion of the reaction a yellow undissolved mass, containing ethyl antimonyl tartrate and silver iodide, is obtained. The mixture is then filtered and washed with absolute alcohol. The residue is then extracted with a small quantity of cold water. The ethyl antimonyl tartrate is obtained from the solution by gently evaporating it on water bath.

Yield—About 2 grams.

It is a white shining powder soluble in water. The solution is distinctly acid to litmus and can be sterilized by gentle boiling without decomposition.

Calculated for $C_2H_5C_4H_4O_7Sb$, Sb = 38·3 per cent.
Found Sb = 37·8 per cent.

Lethal effects obtained from the administration of a one per cent solution of ethyl antimonyl tartrate into guinea-pigs by intramuscular injection :—

TABLE IV

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·05 | 2 | 2 |
| ·045 | 6 | 6 |
| ·04 | 3 | 1 |
| ·03 | 4 | 3 |
| ·02 | 4 | 2 |

Toxicity of the antimony content $= \frac{K}{\cdot 045 \times 37\cdot 8}$ or $\frac{K}{1\cdot 701}$.

Method of preparation of antimonyl tartrates of quinine and cinchonine :—

### (5) *Quinine antimonyl tartrate*

#### EXPERIMENTAL

1·9 grms. of barium antimonyl tartrate are dissolved in 400 c.c. of water and to the solution are added 2·8 grms. of quinine sulphate. The whole is vigorously shaken from time to time for 24 hours, after which it is filtered, when the filtrate is found to be free from sulphate and barium. The filtrate is now kept in the vacuum desiccator for drying. It is very sparingly soluble in water.

Calculated for $C_{24}H_{29}O_9N_2Sb$, Sb = 19·7 per cent. Found Sb = 19·3 per cent. In attempting to prepare quinine antimonyl tartrate by boiling $Sb_2O_3$ with acid quinine tartrate, a portion of the quinine is converted into the toxic quinotoxine.

### (6) *Cinchonine antimonyl tartrate*

#### EXPERIMENTAL

1·18 grms. of cinchonine base is heated on the water bath containing ·6 grm. of tartaric acid until all the cinchonine goes into solution. To the above solution, ·8 grm. of $Sb_2O_3$ is added and the mixture heated on the water bath until all the antimony trioxide goes into solution. The solution is filtered, concentrated and allowed to crystallize. The crystals appear to be globular. They are purified by recrystallization from water.

Calculated for $C_{23}H_{27}O_8N_2Sb$, Sb = 20·7 per cent. Found Sb = 20·6 per cent. The compound can also be prepared in the same way as quinine antimonyl tartrate.

## Experimental

1 grm. of barium antimonyl tartrate is dissolved in water and to this is added a saturated solution of 1·1 grams of cinchonine sulphate. The mixture is slightly heated on the water bath in order to complete the reaction. It is then filtered and the filtrate kept over sulphuric acid and allowed to crystallize, when globules of crystals of cinchonine antimonyl tartrate appear. They are purified by recrystallization.

Calculated for $C_{23}H_{27}O_8N_2$ Sb, Sb = 20·7 per cent.

Found in the dried substance Sb = 20·7 per cent.

Lethal effects obtained from the administration of a solution of quinine antimonyl tartrate into guinea-pigs by intramuscular injection :—

Table V

| Dose in gram per kilo. | No. of guinea pigs used. | Number died. |
|---|---|---|
| ·15 | 3 | 3 |
| ·125 | 5 | 4 |
| ·1 | 6 | 5 |
| ·05 | 2 | *Nil.* |

Toxicity of the antimony content $= \frac{K}{\cdot 15 \times 19{\cdot}7}$ or $\frac{K}{2{\cdot}955}$.

Lethal effects obtained from the administration of a solution of cinchonine antimonyl tartrate into guinea-pigs by intramuscular injection :—

TABLE VI

| Dose in gram per kilo. | No. of guinea-pigs used. | Number died. |
|---|---|---|
| ·15 | 2 | 2 |
| ·125 | 2 | 1 |
| ·1 | 4 | 4 |
| ·09 | 2 | 2 |
| ·08 | 3 | 2 |
| ·07 | 2 | 1 |
| ·06 | 4 | 3 |
| ·05 | 3 | 2 |

Toxicity of the antimony content $= \frac{K}{15 \times 20{\cdot}1}$ or $\frac{K}{3{\cdot}015}$.

## (7) *Narcotine antimonyl tartrate*

Method of preparation :—

### EXPERIMENTAL

4·12 grms. of narcotine are digested with a watery solution of 1·5 grms. of tartaric acid till all the narcotine dissolves. The solution is concentrated and two grms. of antimony trioxide are added and the mixture heated on water bath for some time. The mixture is then diluted with water and filtered. The filtrate is concentrated on the water bath. After cooling, needle-shaped crystals of narcotine antimonyl tartrate are obtained, which are purified by recrystallization. It has no water of crystallization.

Yield—5 grams nearly.

Calculated for $C_{26}H_{28}O_{14}NSb$, Sb = 17·2 per cent.

Found Sb = 16·8 per cent.

Lethal effects obtained from the administration of a solution of narcotine antimonyl tartrate into guinea-pigs by intramuscular injection :—

TABLE VII

| Dose in gram per kilo. | No. of guinea-pigs used. | Number died. |
|---|---|---|
| ·1 | 2 | 2 |
| ·085 | 4 | 4 |
| ·08 | 4 | 3 |
| ·07 | 4 | 2 |
| ·06 | 2 | 1 |
| ·055 | 2 | 0 |
| ·05 | 2 | 0 |

Toxicity of the antimony content $= \frac{K}{\cdot 085 \times 16{\cdot}8}$ or $\frac{K}{1{\cdot}4}$.

## EXPERIMENTS ON RATS

Lethal effects obtained from the administration of a one per cent solution of lithium antimonyl tartrate into rats by intravenous injection :—

TABLE VIII

| Dose in gram per kilo. | No of animals used. | Number died. |
|---|---|---|
| ·03 | 4 | 4 |
| ·025 | 2 | 2 |
| ·02 | 5 | 5 |
| ·015 | 6 | 5 |
| ·01 | 1 | 0 |

Lethal effects obtained from the administration of a one per cent solution of calcium antimonyl tartrate into rats by intravenous injection :—

TABLE IX

| Dose in gram per kilo | No. of animals used. | Number died. |
|---|---|---|
| ·03 | 3 | 3 |
| ·025 | 3 | 1 |
| ·02 | 1 | 1 |
| ·015 | 2 | 1 |
| ·01 | 2 | 0 |

Lethal effects obtained from the administration of a one per cent solution of strontium antimonyl tartrate into rats by intravenous injection :—

TABLE X

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·03 | 2 | 2 |
| ·025 | 1 | 1 |
| ·02 | 2 | 1 |
| ·015 | 1 | 1 |
| ·01 | 1 | 0 |

Lethal effects produced from the administration of a one per cent solution of ethyl antimonyl tartrate into rats by intravenous injection :—

TABLE XI

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·03 | 4 | 4 |
| ·025 | 6 | 5 |
| ·02 | 3 | 2 |
| ·015 | 1 | 0 |

Lethal effects produced from the administration of a solution of quinine antimonyl tartrate into rats by intravenous injection :—

TABLE XII

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·1 | 4 | 4 |
| ·09 | 2 | 1 |
| ·08 | 4 | 3 |
| ·07 | 4 | 1 |
| ·06 | 2 | 1 |

Lethal effects produced from the administration of a solution of cinchonine antimonyl tartrate into rats by intravenous injection :—

TABLE XIII

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·08 | 1 | 1 |
| ·07 | 2 | 2 |
| ·06 | 4 | 3 |
| ·05 | 2 | 0 |

## TOXICITY OF OLD SOLUTIONS OF TARTAR EMETIC AND OF OLD SAMPLES OF STIBENYL

It has been found by many observers that toxic symptoms may follow intravenous injections of old solutions of tartar emetic. This leads one to investigate whether an old solution is more toxic to guinea-pigs and rats than fresh solutions. I give here the results of toxicity experiments with such solutions.

A 2 per cent solution of tartar emetic was kept in an Erlenmeyer flask and the white precipitate that is frequently formed in old solutions was allowed to increase. After three weeks, the solution was made up to the original volume and injected after sterilization along with the precipitate into guinea-pigs and the following results were obtained.

Lethal effects obtained from the administration of an old (three weeks) 2 per cent solution of tartar emetic into guinea-pigs by intramuscular injection :—

TABLE XIV

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·04 | 3 | 3 |
| ·03 | 2 | 1 |
| ·02 | 2 | 1 |
| ·015 | 6 | 0 |

Therefore M. L. D. with guinea-pigs = ·04 grm. per kilo, while in the case of fresh solution M. L. D. = ·055 grm. per kilo. It is thus evident that solutions of tartar emetic become more and more toxic in course of time.

In the case of white rats similar results were obtained as will be seen from the following tables.

Lethal effects obtained from the administration of an old (three weeks) 2 per cent solution of tartar emetic into white rats by intramuscular injection :—

TABLE XV

| Dose in gram per kilo. | No of animals used. | Number died |
|---|---|---|
| ·05 | 2 | 2 |
| ·045 | 2 | 2 |
| ·04 | 9 | 9 |
| ·035 | 4 | 4 |
| ·03 | 3 | 3 |
| ·025 | 3 | 2 |

Lethal effects obtained from the administration of a fresh two per cent solution of tartar emetic into white rats by intramuscular injection :—

TABLE XVI

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·04 | 4 | 4 |
| ·035 | 5 | 3 |
| ·03 | 4 | 3 |
| ·025 | 2 | 1 |
| ·02 | 2 | 0 |

Therefore M. L. D. in the case of white rats with *old solutions* of tartar emetic = ·03 gram per kilo of body weight given intramuscularly, while with fresh solutions it is ·04 per kilo of body weight. Investigations are in progress to determine whether the increase in toxicity is due to any change in the optical activity of the solution.

*Toxicity of Old Samples of Stibenyl*

The first samples of stibenyl that were supplied to me towards the end of 1920 were tested by me after a year and a half and the following results were obtained.

Lethal effects obtained from the administration of a solution of old (nearly a year and a half) sample of stibenyl into guinea-pigs by intramuscular injection :—

TABLE XVII

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·6 | 2 | 2 |
| ·4 | 4 | 4 |
| ·35 | 2 | 1 |
| ·25 | 1 | 1 |

Therefore the M. L. D. of old samples of stibenyl is ·4 gram. per kilo of body weight, while that of fresh samples is ·7 gram per kilo of body weight. It therefore follows that stibenyl kept in powder form becomes more and more toxic in course of time. It may be stated here that no difference in solubility could be observed in these old samples of stibenyl, the substance in each case quickly going into solution.

## Toxicity of Antimonyl Malates

The two antimonyl malates that I have so far investigated are: (1) Ammonium antimonyl malate; (2) Sodium antimonyl malate. In the process of purification of these salts, they are found to crystallize with a molecule of sodium or ammonium hydrogen malate with formation of double salts. Of these salts, I have found that the ammonium salt is more stable, while the sodium salt is easily decomposed in solution on boiling.

## Experimental

*Sodium antimonyl malate.*—About 4 grms. of $Sb_2O_3$ are digested with an aqueous solution of 6·2 grms. of acid sodium malate till no further antimony trioxide is dissolved. The operation is conducted on the water bath under reflux for about an hour when the reaction is complete. The solution is filtered, concentrated and then allowed to crystallize from water.

Yield—6 grams.

*Ammonium antimonyl malate.*—About 4 grms. of $Sb_2O_3$ are digested with 4·5 grms. of acid ammonium malate in the same way as the above, till no more of the $Sb_2O_3$ goes into solution. The process is then conducted in the same way as above. The double salt is purified by recrystallization from water.

Yield—5 grams.

It contains five molecules of water of crystallization.

Calculated for $C_8H_{27}O_{10}N_2Sb$, $Sb = 22 \cdot 7$ per cent.

Found $Sb = 22 \cdot 61$ per cent.

Lethal effects obtained from the administration of a two per cent solution of the double salt of ammonium antimonyl malate and acid ammonium malate into guinea-pigs by intramuscular injection :—

TABLE XVIII

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·125 | 4 | 4 |
| ·09 | 5 | 5 |
| ·08 | 4 | 2 |
| ·075 | 2 | 1 |
| ·07 | 4 | 0 |

Toxicity of the antimony content of the salt $= \dfrac{K}{\cdot 09 \times 22 \cdot 61}$ or $\dfrac{K}{2 \cdot 03}$.

### *Double Salt of Sodium Antimonyl Malate and Acid Sodium Malate*

The toxicity of this salt cannot be determined with accuracy, as it decomposes on boiling during the process of sterilization of its solution.

### TOXICITY OF $Sb_2O_3$ DISSOLVED IN GLYCERINE

Lethal effects obtained from the administration of a two per cent solution of $Sb_2O_3$ in glycerine into guinea-pigs by intramuscular injection :—

TABLE XIX

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·045 | 4 | 4 |
| ·05 | 4 | 4 |
| ·03 | 4 | 4 |
| ·025 | 4 | 1 |
| ·02 | 4 | 0 |

Antimony content of $Sb_2O_3$=83 per cent.

Toxicity of the antimony content= $\frac{K}{\cdot 03 \times 83}$ or $\frac{K}{2\cdot 49}$.

### TOXICITY OF $Sb_2O_3$ DISSOLVED IN TARTARIC ACID

$Sb_2O_3$ gives a series of acids when it combines with tartaric acid. These need not be enumerated here.

In the following experiments, $Sb_2O_3$ was dissolved in the minimum quantity of tartaric acid and the strength of the solution used was in terms of $Sb_2O_3$ dissolved in tartaric acid.

Lethal effects obtained from the administration of a two per cent solution of $Sb_2O_3$ in tartaric acid into guinea-pigs by intramuscular injection :—

TABLE XX

| Dose in gram per kilo. | No. of animals used. | Number died. |
|---|---|---|
| ·035 | 6 | 6 |
| ·03 | 4 | 4 |
| ·025 | 6 | 3 |
| ·02 | 4 | 1 |
| ·015 | 4 | 0 |

Toxicity of the antimony content= $\frac{K}{2\cdot 49}$.

## COMPARISON OF THE TOXICITY OF THE VARIOUS ANTIMONIALS SO FAR INVESTIGATED AND OF THE RESULTS OBTAINED WITH THOSE OF OTHER OBSERVERS

Making a summary of the various antimonials so far investigated by me and calculating on the basis that their toxicity is inversely proportional to their minimum lethal doses, we find that their toxicities in the case of guinea-pigs × 10 are as follows :—

### TOXICITY OF THE ANTIMONY CONTENT OF ANTIMONYL TARTRATES AND MALATES IN THE CASE OF GUINEA-PIGS

| | | | |
|---|---|---|---|
| Quinine antimonyl tartrate | = 1\|30 | Cinchonine antimonyl tartrate | = 1\|30 |
| $Sb_2O_3$ dissolved in glycerine | = 1\|25 | $Sb_2O_3$ dissolved in tartaric acid | = 1\|25 |
| Ammonium antimonyl tartrate | = 1\|23 | Urea antimonyl tartrate | = 1\|21 |
| Strontium antimonyl tartrate | = 1\|20 | Ammonium antimonyl malate | = 1\|20 |
| Potassium antimonyl tartrate | = 1\|20 | Sodium antimonyl tartrate | = 1\|19 |
| Calcium antimonyl tartrate | = 1\|13 | Aniline antimonyl tartrate | = 1\|17 |
| Ethyl antimonyl tartrate | = 1\|17 | Lithium antimonyl tartrate | = 1\|16 |
| Narcotine antimonyl tartrate | = 1\|14 | | |

*The experiments of Farghar and Gray.*—I have not however been able to confirm the observations of these two workers that the toxicity of the antimony content of quinine antimonyl tartrate is only one-fifth that of tartar emetic, though I agree with them that its toxicity is less than that of tartar emetic. I confirm their observations that quinine antimonyl tartrate on boiling with antimony trioxide is converted into the more toxic quino-toxine antimonyl tartrate. I have not been able to confirm their, as well as Rogers', conclusions that the sodium salt is less toxic than the potassium salt. I have confirmed Plimmer and Thompson's observations that the lithium salt is more toxic than the sodium or potassium salt.

I have been able to prepare the antimony analogue of atoxyl without its water of crystallization. Farghar and Gray seem to have obtained the sodium salt of a polymerized derivative of acetyl-*p*-amino-phenyl-stibinic acid, which they could not completely free from the admixture of sodium chloride. By determining the molecular weight of the compound by the freezing point method, after dissolving it in distilled water, it was found that the molecular weight was 266·6, which more nearly corresponds to the constitution of the compound given by me than to that given by Farghar and Gray. My observations on the minimum lethal doses with the antimonyl salts so far investigated on guinea-pigs give higher figures than those obtained by Farghar and Gray in the case of mice.

It would appear from the observations of the above workers that the least toxic antimonyl tartrate to be used in the case of kala-azar should be quinine antimonyl tartrate. The effective dose of tartar emetic in the case of kala-azar is generally 5 c.c. of a two per cent solution (= ·1 grm. or 1·5 grains). According to their observations 5 times the effective dose of tartar emetic can be used in the case of quinine antimonyl tartrate. This will be 7·5 grains. The amount of quinine base present in 7·5 grains of quinine antimonyl tartrate is nearly 4 grains. Apart from any other effect of quinine, the intravenous injection of 4 or 5 grains of quinine in a concentrated solution given rapidly may sometimes lead to a dangerous fall of blood pressure, especially in a weak and debilitated kala-azar patient and therefore should not be advocated. I have discussed the effect of the fall of blood pressure after intravenous injection of quinine elsewhere.

Amongst others who have worked on the toxicity of antimony compounds may be mentioned Carl Voegtlin and co-workers who experimented with albino rats. Korns experimented with rabbits.

## (B)
## DELAYED ANTIMONY POISONING

In some rare cases, after intramuscular injection of an antimonyl tartrate or malate into guinea-pigs, I have found that death took place even so late as three weeks or more after one injection. The viscera which showed pathological changes were subsequently subjected to chemical examination and showed the presence of antimony. The organs examined were the liver, the lungs and the kidneys. These cases may be described as cases of *delayed antimony poisoning*.

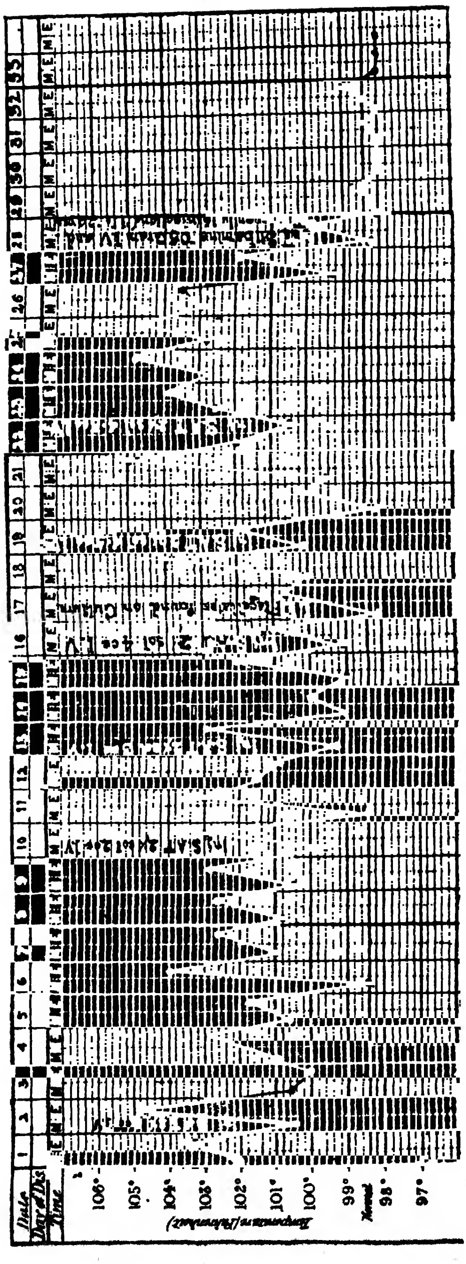
The details of *post-mortem* examination of a number of guinea-pigs that died of delayed antimony poisoning are as follows :—

(1) *Serial No.* 449.—Guinea-pig weighing 237 grms. was given an intramuscular injection of a two per cent solution of tartar emetic in dose of ·04 grm. per kilo of body weight. Total quantity of tartar emetic injected was ·0095 gram. The animal died 22 days after the injection. On *post-mortem* examination, there were hæmorrhages in both the lungs. Liver was fatty and there were patches of necrosis here and there over the surface of the liver. Kidneys were congested. The animal evidently died of antimony poisoning, the viscera showing the presence of antimony.

(2) *Serial No.* 445.—Guinea-pig weighing 455 grms. was given an intramuscular injection of a two per cent solution of sodium antimonyl tartrate in dose of ·04 grms. per kilo of body weight. Total quantity of sodium antimonyl tartrate injected was ·018 grm. The animal died 14 days after the injection. On *post-mortem* examination there were hæmorrhages in the gall bladder. The kidneys were congested. The animal evidently died of antimony poisoning, the viscera showing the presence of antimony.

(3) *Serial No.* 426.—Guinea-pig weighing 620 grms. was given an intramuscular injection of a two per cent

Serial No. 15 (*a*)



Temperature chart of a kala-azar case treated successfully with urea stibamine.

solution of double salt of ammonium antimonyl malate and acid ammonium malate in dose of ·5 grm. per kilo of body weight. The total quantity of ammonium antimonyl malate injected was ·262 grm. The animal died three weeks after the injection. On *post-mortem* examination there were hæmorrhages in both the lungs. The liver was fatty and the kidneys congested. The animal evidently died of antimony poisoning, the viscera showing the presence of antimony.

(4) *Serial No.* 439.—Guinea-pig weighing 495 grms. was given an intramuscular injection of one per cent solution of tartar emetic in dose of ·5 grm. per kilo of body weight. The total quantity of tartar emetic injected was ·025 grm. The animal died 19 days after the injection. On *post-mortem* examination there were hæmorrhages in the left lung. There was a catarrhal condition of the whole gastro-intestinal tract. Liver was fatty and there were patches of necrosis on the surface. Kidneys were congested. Traces of antimony were found in the liver, the lungs, the kidney and the intestines. Animal died of antimony poisoning.

(5) *Serial No.* 432.—Guinea-pig weighing 160 grms. was given an intramuscular injection of a one per cent solution of tartar emetic in dose of ·04 grm. per kilo of body weight. Total quantity of tartar emetic injected was ·0064 grm. The animal died three weeks after the injection. On *post-mortem* examination there were hæmorrhages in the right lung. The gastro-intestinal tract was ulcerated. The liver was fatty and there were patches of necrosis on its surface. The kidneys were congested. On chemical examination of the liver, the kidneys, the lungs and the intestines, there was distinct presence of antimony. The animal died of antimony poisoning.

(6) *Serial No.* 463.—Guinea-pig weighing 200 grms. was given an intramuscular injection of a one per cent solution of sodium antimonyl tartrate in dose of ·045 grm.

per kilo of body weight. The total quantity of sodium antimonyl tartrate injected was ·009 grm. The animal died 18 days after the injection. On *post-mortem* examination, there were hæmorrhages in the right lung. The liver was fatty and there were patches of necrosis on the surface. The kidneys were congested. There were distinct traces of antimony in the viscera which showed the pathological changes. The animal died of antimony poisoning.

(7) *Serial No.* 450.—Guinea-pig weighing 227 grms. was given an intramuscular injection of a one per cent solution of tartar emetic in dose of ·04 grm. per kilo of body weight. The total quantity of potassium antimonyl tartrate injected was ·0091 grm. The liver was fatty. The kidneys were pale and somewhat enlarged in size. There was distinct presence of antimony in the liver, the kidneys and the lungs. The animal died of antimony poisoning 26 days after injection.

## Method of Chemical Examination of Viscera for Detection of Antimony

The organs are cut into small pieces with a pair of scissors. Then these are digested under slow heat with chemically pure HCl (1 in 4), to which a piece of bright copper foil, free from arsenic or antimony, is added. If arsenic or antimony is present, then there is a deposit on the copper. The piece of copper is then washed with alcohol and then with some ether and subsequently made absolutely dry. Then it is put into a hard glass reduction tube and heated for some time. The sublimate obtained on the cold portion of the tube is examined under the microscope, and if antimony is present then an amorphous deposit or sometimes characteristic needle-shaped crystals of $Sb_2O_3$ are obtained. If the sublimate is sufficient, it can then be dissolved in dilute HCl and $H_2S$ passed into the solution. An orange coloured precipitate shows the presence of Sb in the solution.

Cases of delayed antimony poisoning are of very great clinical importance, as they prove that the excretion of the drug may sometimes be very slow after injection of antimonial compounds and some of the cases of sudden death during antimonial treatment may be due to a cumulative action of the drug.

## Remarks

1. The toxicity of the following antimonial compounds has been worked out in the present paper: (1) Lithium antimonyl tartrate, (2) Calcium antimonyl tartrate, (3) Strontium antimonyl tartrate, (4) Ethyl antimonyl tartrate, (5) Quinine antimonyl tartrate, (6) Cinchonine antimonyl tartrate, (7) Narcotine antimonyl tartrate, (8) Ammonium antimonyl malate, (9) $Sb_2O_3$ dissolved in glycerine, and (10) $Sb_2O_3$ dissolved in tartaric acid. By comparing their toxicities it was found that quinine antimonyl tartrate is one of the least toxic antimonial salts. But for reasons that quinine is likely to bring about a dangerous fall of blood pressure, after an intravenous injection, its antimonyl tartrate cannot be recommended for use in place of ammonium, sodium or potassium antimonyl tartrates. $Sb_2O_3$ dissolved in glycerine or tartaric acid comes next in order of low toxicity, but for obvious reasons, the solution cannot be recommended for use intravenously. $Sb_2O_3$ dissolved in tartaric acid is the basis of the author's hyperacid antimonyl tartrate which has given satisfactory results in the treatment of kala-azar when used *intramuscularly*. $Sb_2O_3$ dissolved in glycerine is the basis of Martindale's *injectio antimonii oxidi*.

2. Old solutions of tartar emetic and old samples of stibenyl have been found to be more toxic than fresh ones.

3. Cases of death in guinea-pigs three weeks or so after one injection of an antimonial salt have been met with, showing definite symptoms of antimony poisoning and presence of antimony in the viscera.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, December 19, 1922]

## PART IV

### FURTHER OBSERVATIONS ON THE THERAPEUTIC VALUE OF UREA STIBAMINE

New series of cases :

(1) Patient, named Puran, æt. 12 years, was admitted into hospital with history of double rise of temperature. Spleen extended 7 inches below the costal margin in the left nipple line. Body weight—3 st. 2 lbs. Patient was treated with intravenous injection of urea stibamine twice a week. During the treatment patient had frequent attacks of dysentery. Fever stopped after five injections. Altogether 20 injections were given, each dose being ·1 gram and the duration of treatment four months. Patient was under observation for nearly a month after treatment was stopped during which there was no fever and at the time of discharge, the spleen could not be felt below the costal margin nor could L. D. bodies be found on spleen puncture. Body weight—3 st. 8 lbs.

| Blood count. | Spleen puncture. | REMARKS. |
|---|---|---|
| R. B. C.—2,100,000, W. B. C.—1,800, Hb—32% | Positive | Before treatment. |
| R. B. C.—4,200,000, W. B. C.—6,200, Hb—54% | Negative | 120 days after commencement of treatment. Total amount injected —2 grams in 20 injections after which treatment was discontinued. |
| R. B. C.—4,200,000, W. B. C.—7,800, Hb—52% | Negative | 26 days after treatment was discontinued when patient was discharged. |

(2) Patient, named Jatin, æt. 25 years, was admitted into hospital with double rise of temperature. Spleen—7½ inches below the costal arch. Body weight—5 st. 4 lbs. Patient was treated with intravenous injection of urea stibamine twice a week in doses of 5 c.c. to 7½ c.c. of a 2 per cent solution (= ·1 to ·15 grm.). As a result of treatment the fever stopped after three injections. Patient was under observation for nearly a month after the injections were stopped and at the time of discharge spleen could not be felt below the costal margin. Body weight—6 stone.

| Blood count. | Spleen puncture. | REMARKS. |
|---|---|---|
| R. B. C.—2,300,000, W. B. C.—1,400, Hb—36% | Positive | Before treatment. |
| R. B. C.—2,900,000, W. B. C.—2,400, Hb—44% | ... | 18 days after treatment was continued, 6 injections having been given. Amount injected—·8 grm. |
| R. B. C.—3,800,000, W. B. C.—4,200, Hb—48% | Negative | 48 days after commencement of treatment, 12 injections having been given, after which the injections were discontinued. Amount injected—1·7 grms. |
| R. B. C.—3,800,000, W. B. C.—5,400, Hb—52% | Negative | 35 days after treatment was discontinued, when the patient was discharged. |

(3) Patient, named Bepin, was admitted into the hospital for treatment of kala-azar with continuous fever. Spleen—4 inches below costal arch. Body weight—6 st. 7½ lbs. Patient was treated with intravenous injection of urea stibamine twice a week in doses of 5 c.c. of a 2 per cent solution (= ·1 grm.). There was slight rise of temperature with rigor after the first five injections. The fever came down to

normal after the sixth injection. The spleen could not be felt below the costal margin when the treatment was stopped. The patient was under observation for nearly three weeks after the injections were stopped, during which there was no fever and at the time of discharge no L. D. bodies could be found on spleen puncture. Body weight—7 stone.

| Blood count. | Spleen puncture. | REMARKS. |
|---|---|---|
| R. B. C.—3,100,000, W. B. C.—1,400, Hb—44% | Positive | Before treatment. |
| R. B. C.—2,900,000, W. B. C.—2,800, Hb—40% | ... | 20 days after commencement of treatment, 6 injections having been given. Amount injected—·55 grm. |
| R. B. C.—3,400,000, W. B. C.—6,400, Hb—52% | Negative | 63 days after commencement of treatment, 16 injections having been given, after which they were discontinued. Amount injected—1·55 grms. |
| R. B. C.—3,400,000, W. B. C.—7,000, Hb—52% | Negative | 24 days after treatment was discontinued, when the patient was discharged. |

(4) Patient, named Abdul, æt. 20 years, was admitted into the hospital for treatment of kala-azar with fever. Spleen—3½ inches below costal arch. Spleen puncture—L. D. bodies found. Body weight—4 st. 18 lbs. He was treated with intravenous injection of urea stibamine in doses of 5 c.c. to 7½ c.c. of a 2 per cent solution (=·1 grm. to ·15 grm.). There was slight nausea without any other reaction after each injection. The spleen could not be felt below the costal margin shortly after the completion of injection. He was 30 days under observation after injections were stopped, during which there was no rise of temperature. At the time of discharge no L. D. bodies could be

determined on spleen puncture. Body weight—5 st. 2 lbs. Duration of treatment—42 days.

| Blood count. | Spleen puncture. | REMARKS. |
|---|---|---|
| R. B. C.—2,900,000, W. B. C.—1,600, Hb—40% | Positive | Before treatment. |
| R. B. C.—3,400,000, W. B. C.—3,200, Hb—42% | ... | 30 days after commencement of treatment, 10 injections having been given. Amount injected—1 grm. |
| R. B. C.—3,400,000, W. B. C.—5,400, Hb—46% | Negative | 42 days after commencement of treatment, 15 injections having been given, after which they were stopped. Amount injected—1·75 grams. |
| R. B. C.—3,400,000, W. B. C.—6,200, Hb—50% | Negative | 30 days after treatment was discontinued, when patient was discharged. |

(5) Patient, named Prithi Raj, æt. 30 years, was admitted into hospital with very high fever and slightly enlarged spleen. At the time of admission the patient was in a drowsy state which lasted for 4 days after admission, the spleen rapidly increasing to 3½ inches below the costal arch. The blood showed no malarial parasites. Body weight after he recovered from his drowsiness—6 stone. Patient used to get rise of temperature with rigor. L. D. bodies found on spleen puncture. The patient was treated with intravenous injection of urea stibamine in doses of 5 c.c. to 7½ c.c. of a 2 per cent solution (=·1 to ·15 grm.). The fever stopped after three injections. Very few L. D. bodies were found on spleen puncture after six injections. Altogether 15 injections were given and after this the spleen could just be felt below the costal arch; at the time of discharge no L. D. bodies could be found on spleen puncture. Duration of treatment—66 days.

| Blood count. | Spleen puncture. | REMARKS. |
|---|---|---|
| R. B. C.—3,600,000, W. B. C.—1,400, Hb—48% | Positive | Before treatment. |
| R. B. C.—4,300,000, W. B. C —6,400,Hb—50% | Negative | 66 days after commencement of treatment, altogether 15 injections having been given. Total amount injected—2 grms. |
| R. B. C.—4,500 000, | Negative | 40 days after completion of treatment. |
| W. B. C.—6,800, Hb—52% | Negative | 60 days after treatment was stopped, after which patient was discharged from hospital in excellent condition. |

(6) Patient, named Monglu, æt. 18 years, was admitted into hospital suffering from kala-azar. Spleen—4 inches below costal arch. Body weight—3 st. 4 lbs. There was slight jaundice. L. D. bodies were found on spleen puncture. Patient was treated with intravenous injection of urea stibamine in doses of 5 c.c. to 7½ c.c. of a 2 per cent solution (= ·1 to ·15 grm.). Altogether 20 injections were given. He did not get much reaction after the injections, except nausea after injection of 7½ c.c. of a 2 per cent solution. As a result of treatment the fever subsided. At the time when the injections were discontinued the spleen did not much diminish in size, but no L. D. bodies could be found on spleen puncture. Patient remained in hospital for 60 days after the injections were stopped, and at the time of discharge the spleen completely disappeared below the costal arch and the patient improved considerably in health. Body weight—4 stone. Duration of treatment—95 days.

| Blood count. | Peripheral blood culture N.N.N. medium | Spleen puncture | Spleen blood culture N.N N. medium | REMARKS |
|---|---|---|---|---|
| R. B. C.—2,500,000, W. B. C.—1,800, Hb—36% | Positive | Positive | Positive | Before treatment. |
| R B. C.—4,500,000, W. B. C.—4,800, Hb—52% | ... | ... | ... | 46 days after commencement of treatment, 10 injections having been given. Total amount—·95 grm. |
| R. B. C.—4,500,000, W. B. C.—5,800, Hb—58% | Negative | Negative | Negative | 95 days after commencement of treatment, 20 injections having been given, after which treatment was stopped. Total amount—2·35 grms. |
| | Negative | Negative | Negative | 35 days after completion of treatment. |
| R. B. C. 4,400,000, W. B C.—5,800, Hb—58% | Negative | Negative | Negative | 55 days after completion of treatment. Patient left hospital 60 days after completion of treatment. |

(7) Patient, named Romesh, æt. 14 years, was admitted into the hospital suffering from kala-azar with fever and dysentery and oedema of the extremities. Spleen 6 inches below costal arch. L.D. bodies found on spleen puncture. Body weight 3 stone. Patient was treated with intravenous injection of urea stibamine in doses of $2\frac{1}{2}$ c.c. to 5 c.c. of a 2 per cent solution (= ·05 to ·1 grm.) in spite of dysentery. Developed *cancrum oris* after eight injections which healed up in course of treatment. As a

result of treatment the fever subsided and when the treatment was discontinued, spleen extended $2\frac{1}{2}$ inches below the costal arch, but no L. D. bodies could be found on spleen puncture. Patient remained in hospital for 70 days after the injections were stopped, and at the time of discharge the spleen completely disappeared under the costal arch. Body weight—4 st. 2 lbs. Duration of treatment—90 days.

| Blood count | Peripheral blood culture. N.N.N. medium | Spleen puncture | Spleen blood culture N.N.N. medium | REMARKS |
|---|---|---|---|---|
| R. B. C.—2,600,000, W.B C.—1,200, Hb—32% | Positive | Positive | Positive | Before treatment. |
| R. B. C.—4,100,000, W B.C.—6,600, Hb—52% | ... | ... | ... | 52 days after commencement of treatment, 12 injections having been given. Total amount —1·15 grms. |
| R. B. C.—3,700,000, W.B.C.—6,400, Hb—52% | Negative | Negative | Negative | 90 days after commencement of treatment, 20 injections having been given after which the injections were stopped. Total amount — 1·95 grms. |
| ... | Negative | Negative | Negative | 33 days after completion of treatment. |
| R. B. C.—3,600,000, W.B.C.—7,000, Hb—48% | Negative | Negative | Negative | 65 days after completion of treatment, after which the patient left hospital. |

(8) Patient, named Phaninder, æt. 15 years, was admitted into the hospital for treatment of kala-azar. Spleen—4½ inches below costal arch. Temperature varied from normal to 103°F. Bleeding from the gums and nose present. Body weight—5 st. 4 lbs.* No L. D. bodies found on spleen puncture. Patient was treated with intravenous injection of urea stibamine in doses of 5 c.c. to 7½ c.c. of a 2 per cent solution (= ·1 to ·15 grm.). No reactions after the injections. Altogether 16 injections were given. As a result of treatment the fever stopped after 12 injections, when the spleen could hardly be felt below the costal arch. After 16 injections, no L. D. bodies could be found on spleen puncture. Patient is still in hospital, 90 days after completion of treatment. Body weight—6 stone. Duration of treatment—50 days.

| Blood count | Peripheral blood culture N.N.N. medium | Spleen puncture | Spleen blood culture N.N.N. medium | REMARKS |
|---|---|---|---|---|
| R. B. C.—2,300,000, W. B. C.—2,600, Hb—36 % | Positive | Negative | Positive | Before treatment. |
| R. B. C.—3,700,000, W. B. C.—3,200, Hb—48 % | Negative | Negative | Positive | 16 days after commencement of treatment, 6 injections having been given. Total amount —·45 grm. |
| R. B. C.—3,500,000, W. B. C.—4,200, Hb—44 % | Negative | Negative | Negative | 50 days after commencement of treatment, 16 injections having been given, after which treatment was stopped. Total amount — 1·65 grms. |

* *The case is interesting as no L. D. bodies could be found on spleen puncture from the beginning but positive results were obtained by culture.*

| Blood count. | Peripheral blood culture N.N.N. medium. | Spleen puncture. | Spleen blood culture N.N.N. medium. | REMARKS. |
|---|---|---|---|---|
| R. B. C.—5,000,000, W. B. C. –8,200, Hb—50 % | Negative | Negative | Negative | 120 days after completion of treatment, patient still in hospital. His general condition shows remarkable improvement. |

(9) Patient, named Haren, æt. 12 years, was admitted into the hospital with spleen extending $5\frac{1}{2}$ inches below costal arch. Many L. D. bodies found on spleen puncture. Body weight—3 st. 3 lbs. Temperature ranged from 100°F. to 102°F. Bleeding from the gums and nose present. Patient was treated with intravenous injection of urea stibamine, the dose being 5 c.c. of a 2 per cent solution (= ·1 grm.). During treatment patient developed *cancrum oris*. Temperature began to come down after four injections and after six injections it remained permanently normal. Altogether 13 injections were given after which the spleen could hardly be felt below costal arch. Patient is still in hospital, 80 days after completion of treatment. Increase of body weight—1 stone. Patient's general condition—very satisfactory. Duration of treatment—43 days.

| Blood count. | Peripheral blood culture N.N.N. medium. | Spleen puncture. | Spleen blood culture N.N.N. medium. | REMARKS. |
|---|---|---|---|---|
| R. B. C.—2,800,000. W. B. C —1,000, Hb—42 % | Positive | Positive | Positive | Before treatment. |
| R. B C.—3,200,000, W. B C.—3,600, Hb –46 % | Positive | Positive | Positive | 18 days after commencement of treatment, 6 injections having been given Total amount —·6 grm. |

| Blood count. | Peripheral blood culture N.N.N. medium. | Spleen puncture. | Spleen blood culture N.N.N. medium. | REMARKS. |
|---|---|---|---|---|
| R. B. C.—4,200,000, W. B. C.—6,400, Hb—48% | Negative | Negative | Negative | 32 days after commencement of treatment, 10 injections having been given. Total amount —1 grm. |
| R. B. C.—4,600,000, W. B. C.—7,000, Hb—48% | Negative | Negative | Negative | 43 days after commencement of treatment, 13 injections having been given, after which they were stopped. Total amount — 1·4 grms. |
| R. B. C.—4,600,000, W. B. C.—9,000, Hb—52% | Negative | Negative | Negative | 53 days after completion of treatment patient is still in hospital. General conditions — very satisfactory. |

*Further Notes on Cases Previously Reported in the Indian Journal of Medical Research, October, 1922*

No. I. Patient Monu, left hospital 75 days after treatment was stopped. At the time of discharge no L. D. bodies were found on spleen puncture. Spleen could not be felt below the costal arch. Increase in weight—1 stone. No fever since treatment was stopped. General condition—very satisfactory. R. B. C.—4,200,000, W. B. C.—8,600, Hb—54% at the time of discharge.

No. II. Patient, Tofu, left hospital 51 days after treatment was stopped. At the time of discharge no L. D.

bodies were found on spleen puncture. Spleen could not be felt below the costal arch. No fever since treatment was stopped. General conditon very satisfactory. R. B. C.—4,400,000, W. B. C.—10,200, Hb—60% at the time of discharge.

No. IV. Patient, Anath Bandhu, left hospital 50 days after treatment was stopped. At the time of discharge no L. D. bodies were found on spleen puncture. Spleen could not be felt below the costal arch. No fever since treatment was stopped. General condition very satisfactory. Increase of weight—9 lbs. during 30 days after completion of treatment. R. B. C.—3,400,000, W. B. C.—8,200, Hb—58%.

No. VII. Patient, Horoz, is still in hospital, *i.e.*, 10 months after treatment was stopped. Cultural reports are attached herewith. General condition very satisfactory. No fever since the treatment was stopped.

| Blood count. | Peripheral blood culture N.N.N. medium. | Spleen puncture. | Spleen blood culture N.N.N. medium. | REMARKS. |
|---|---|---|---|---|
| R. B. C.—4,800,000, W. B. C.—7,200, Hb—52% | Negative | Negative | Negative | 130 days after treatment was stopped. |
| R. B. C—5,100,000, W. B C.—8,600, Hb—60% | Negative | Negative | Negative | 305 days after treatment was stopped. Patient is still under observation in hospital, in very excellent condition of health. |

*Remarks.*—Case No. VII, Horoz, has been kept in hospital for a prolonged period to test the permanency of the efficiency of treatment. The remaining four cases reported in

my first paper and not reported here left hospital soon after my first paper was sent for publication.

The method adopted for the culture of *L. donovani* in my experiments is as follows (Major Knowles, I.M.S.); it is a modification of Row's method :—

About a quarter of a c.c. of blood from a vein at the bend of the elbow is put into 20 c.c. of citrated salt solution (normal saline containing 1·5 per cent sodium citrate) : the mixture is shaken gently and allowed to stand for some time. As soon as the corpuscles have settled to the bottom of the tube, the supernatant fluid is poured off and the corpuscles are pipetted off with a capillary pipette and inoculated into the water of condensation at the bottom of N.N.N. tubes which are then incubated at 22°C.

In the case of splenic blood, the syringe is filled with a few drops of the citrate solution mentioned above and then the spleen is punctured. The blood drawn is then mixed with the citrate saline inside the syringe, transferred to the N.N.N. medium and incubated in the same way as in the case of peripheral blood.

In an untreated case, the parasites flagellate generally within the first week.

*N.B.*—To ensure a successful result, strict aseptic precautions are necessary, as even slight bacterial contamination kills the L. D. parasites though they have been found to grow luxuriantly with fungi.

I think Frankel is not justified in stating in his "Die Arzentimittel Synthese" that changes in the molecular structure of antimony compounds do not bring about an increase of their therapeutic properties. Urea stibamine is just as useful in the treatment of kala-azar as atoxyl or soamin in diseases for which they have been recommended.

## Remarks

A series of cases have been described which have been cured by the intravenous injection of urea stibamine.

My grateful thanks are due to Major Knowles, I.M.S., Protozoologist, Calcutta School of Tropical Medicine, for helping me in making the cultural tests in his laboratory. I am also indebted to my staff, Mr. Saroda Churan Chowdhury, M.Sc., Mr. Judhistir Dass, M.Sc., Sub-Asst. Surgeons, Bibhuty Bhushan Maity and Sirish Chandra Banerjee, for their faithful services and hearty co-operation in helping me in carrying on my researches.

Through the kind courtesy of Major H. E. Shortt, I.M.S., Assam Scientific Research Committee, Shillong, I am appending here an extract from his notes on the use of urea stibamine :—

## Extract from a Note on the Use of Urea Stibamine

BY

Major H. E. SHORTT, I.M.S.
*Assam Scientific Research Committee, Shillong*

Five cases altogether were treated with this preparation and, in our opinion, with most encouraging results. As the results obtained in some at least of these cases were very striking, the particulars of each case will be mentioned in some detail in the annexed table. The dosage employed by us was that recommended by Dr. Brahmachari, the solution for each injection being made up afresh. The initial dose was 0·1 gram dissolved in cold sterile distilled water. Each subsequent dose, given on alternate days, reached 0·25 gram which was not exceeded. Thus the fourth and all subsequent doses were of 0·25 gram. As the preparation is precipitated from alcohol, it is presumably sterile

and on solution in cold sterile distilled water needs only warming in a water bath. Administration was made by intravenous route. Results of spleen puncture were tested by microscopic and cultural methods. The details of the cases treated are given below in tabular form. The perusal of the table will at once show that..............the results obtained were so favourable as to encourage one to make a further extensive trial of this preparation. Its advantages over the antimony preparations usually employed as evidenced by experience of it in these five cases are :

(1) The short course, occupying only two to three weeks, necessary to a complete cure.

(2) The rapidity with which the symptoms of the disease disappear.

(3) The fact that no symptoms of intolerance were met with in any of the cases.

The results here recorded are actually better than those claimed by Dr. Brahmachari (1922) himself in the published account of some of his cases, where he gave as many as 20 injections.

## TABLE

*Showing Results of Cases Treated with Urea Stibamine*

| Case No. | Age. | Duration of illness on admission. | Result of spleen puncture. | Amount of urea stibamine after which spleen puncture was negative. | No. of injections after which spleen puncture was negative. | Total amount of urea stibamine administered. | Weight in pounds before and after treatment. | Result. | REMARKS. |
|---|---|---|---|---|---|---|---|---|---|
| 43 | 22 | 11 months | +++ | ·95 gramme | 5 | 1·7 grammes | 117½ – 133 | cure | Case on admission was very ill with marked œdema of legs and great weakness. |
| 48 | 17 | 11½ months | +++ | 1·7 grammes | 8 | 1·7 grammes | 78–99 | cure | Case on admission weak with œdema of feet. Spleen puncture was not repeated before the eighth injection. |
| 56 | 15 | 6 months | +++ | ·7 gramme | 4 | 1·7 grammes | 56–71½ | cure | Case on admission, emaciated, weak and very anæmic. |
| 57 | 40 | 9½ months | +++ | 1·795 grammes | 9 | 2 295 grammes | 93 – 110 | cure | Case on admission was extremely weak with severe bronchitis. Parasites very numerous. |
| 58 | 38 | 12 months | ++ | ·7 gramme | 4 | Still under treatment. | ... | ... | Case had previously received full treatment with sodium antimonyl tartrate without benefit. |

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, December 19, 1922]

## PART V

## AMINO-ANTIMONYL TARTRATES

### *General Method of Preparation of the New Amino-Antimonyl Tartrates*

(1) In the first method, molecular proportions of tartaric acid and the base were dissolved in boiling water. To this solution were subsequently added molecular proportions of antimony trioxide. The whole was heated till the oxide went into solution. The solution was then filtered and the filtrate solidified by gentle heating. The product was purified by repeated crystallization from water. In some cases the acid tartrate of the base was boiled gently with antimony trioxide.

(2) In the second method, molecular proportions of tartar emetic or sodium antimonyl tartrate and the hydrochloride of the base were refluxed in a medium of alcohol and water (6 to 2) for an hour. The solution was filtered and the filtrate was then gently heated till a solid mass was obtained. The salt was purified from sodium or potassium chloride by repeated crystallization from water and alcohol. The following reaction takes place :—

$$C_4H_4O_6\,SbOK + R.\,HCl = C_4H_5O_6SbO\,R + KCl.$$

(3) In the third method, molecular proportions of silver antimonyl tartrate and the hydrochloride of the base were either refluxed in a medium of water for an hour or kept at the ordinary temperature for a day with frequent and vigorous agitation. When the reaction was complete the solution was filtered and the filtrate concentrated, either in the water bath or *in vacuo*, when a solid or semi-solid mass resulted. In the first case, the mass was purified by recrystallization from water, while in the second case the semi-solid mass became solid on treatment with absolute alcohol. This solid mass was purified by dissolving in water and subsequent precipitation with alcohol or with alcohol and acetone. The following reaction takes place :—

$$C_4H_4O_6 \,.\, OSbAg + R \,.\, HCl = C_4H_5O_6 \,.\, OSb \,.\, R + AgCl.$$

Acriflavine antimonyl tartrate was prepared in a special way to be described below :—

### (*1*) *Preparation of p-phenetidinyl-acetamido-antimonyl-tartrate* (=*phenocoll antimonyl tartrate*)

1·5 grms. of tartaric acid were dissolved in water and to the solution were added phenocoll base 2 grms. and the whole was heated till the base went into solution completely. Antimony trioxide 2 grms. were gradually added to it and the whole was boiled for 15 minutes. It was then filtered, the filtrate being concentrated and the oil separated. This oil on cooling and after agitation solidified into a crystalline mass. This was then purified by double recrystallization from water.

Yield—almost theoretical.

Calculated for $C_{14}H_{19}O_9N_2Sb$, Sb=25·06%. Found Sb=25·08%.

*This compound has been prepared with the idea of using it intramuscularly.*

### (2) *Preparation of p-carbethoxy-aniline-antimonyl-tartrate(=anæsthesin antimonyl tartrate)*

1·5 grms. of tartaric acid were dissolved in water and to the solution were added 1·6 grms. of the ethyl ester of *p*-amino-benzoic acid and excess of water; the mixture was then boiled until the whole mass went into solution. To this solution was added gradually antimony trioxide in excess (nearly 1·6 grms.) and the whole was boiled for half an hour. It was filtered and the filtrate on concentration and cooling produced a crystalline mass. It was dissolved in water under reflux and allowed to cool, when well-defined crystals were obtained. (Yield = 2 grams.).

Calculated for $C_{13}H_{16}O_9$ Sb. Sb = 26·66%. Found Sb = 26·6%.

Properties.—It is not very freely soluble in water, but its solubility is markedly increased in water containing a trace of tartaric acid.

*This compound was prepared with the idea of using it internally, as anæsthesin has a marked anæsthetic effect on the stomach.*

### (3) *Preparation of novocaine antimonyl tartrates*

Two antimonyl tartrates have been obtained from novocaine. In the first, one molecule of antimonyl tartaric acid combined with novocaine base, while in the second two molecules of antimonyl tartaric acid combined with the base.

*These compounds have been prepared with the idea of using them intramuscularly.*

#### (*a*) *Preparation of novocaine-mono-antimonyl tartrate*

0·546 grm. of novocaine hydrochloride was dissolved in 20 c.c. of water in a 50 c.c. flask and to this was added 0·82 grm. of silver-antimonyl tartrate. The flask was well corked,

protected from sunlight by non-actinic paper and frequently vigorously shaken. It was kept in this condition for six days after which it was filtered. The filtrate was tested and found free from chlorine and silver. The filtrate was allowed to concentrate in a vacuum desiccator. On concentration the solution set to a gel which on treatment with alcohol yielded a white solid. This was quickly collected, washed with ether, and again dried in a vacuum desiccator.

Calculated for $C_{17}H_{25}O_9N_2$ Sb, $(C_2H_5)_2$ $CH_5O_6$ (SbO), Sb = 23·03%. Found Sb = 23%. Yield = ·3 grm.

It is highly hygroscopic and is easily soluble in water.

(*b*) *Preparation of novocaine-di-antimonyl tartrate*

1·5 grams. of tartaric acid were dissolved in 30 c.c. of water by heating. To the solution were added 1·2 grms. of novocaine base and then gradually 1·6 grms. of antimony trioxide while the whole was kept boiling for half an hour after addition of antimony trioxide. The unattacked antimony trioxide was separated by filtration, the filtrate concentrated and absolute alcohol added to the solution, when a white crystalline highly hygroscopic substance was obtained. This was next treated with alcohol and acetone and subsequently kept in a vacuum desiccator over sulphuric acid.

Calculated for $C_{21}H_{30}O_{16}N_2Sb_2$, Sb = 29·7%. Found Sb = 29·2%.

Novocaine-di-antimonyl tartrate is highly hygroscopic and undergoes decomposition when kept in a wet condition in the air, yielding a yellow substance. It is very soluble in water.

(*4*) *Preparation of apothesine antimonyl tartrate*

EXPERIMENTAL

1·2 grms. of apothesine hydrochloride are dissolved in water and to the solution are added 1·64 grms. of silver anti-

monyl tartrate. After 48 hours, during which it is frequently and vigorously shaken, the mixture is filtered when the filtrate was found to be free from chlorine and silver. It is then placed in a vacuum desiccator and on concentration a jelly-like mass was obtained which solidified on treatment with absolute alcohol. It is collected and washed with absolute alcohol and acetone and dried in a vacuum desiccator. (Yield = 0·5 gram.)

Calculated for $C_{20}H_{28}O_9NSb$, Sb = 21·97%. Found Sb = 21·96%.

The salt is highly hygroscopic and is easily soluble in water.

*The compound was prepared with the idea of using it intramuscularly.*

The toxicity of two of the above antimonyl tartrates is given below :—

Lethal effects obtained from the administration of a one per cent solution of phenocoll-antimonyl tartrate into guinea-pigs by intramuscular injection.

TABLE I

| Dose in grams per kilo. | No. of animals used. | Number died. |
|---|---|---|
| 0·08 | 3 | 3 |
| 0·075 | 3 | 2 |
| 0·07 | 3 | 2 |
| 0·065 | 3 | 2 |
| 0·055 | 1 | 0 |

Toxicity of the antimony content $= \frac{K}{0{\cdot}08 \times 25{\cdot}06}$ or $\frac{K}{2{\cdot}0048}$.

Lethal effects obtained from the administration of a one per cent solution of anæsthesin-antimonyl tartrate into guinea-pigs by intramuscular injection.

TABLE II

| Dose in grams per kilo. | No. of animals used. | Number died. |
|---|---|---|
| 0·09 | 3 | 3 |
| 0·085 | 2 | 1 |
| 0·08 | 1 | 0 |

Toxicity of the antimony content $= \frac{K}{\cdot 09 \times 26\cdot 6}$ or $\frac{K}{2\cdot 394}$.

(5) *Preparation of methyl-amino-hydroxybenzoate antimonyl tartrate (=orthoform antimonyl tartrate)*

EXPERIMENTAL

1·5 grms. of tartaric acid dissolved in 50 c.c. of water are heated with 1·7 grms. of 3-amino-4-hydroxy-methyl benzoic ester till the latter goes into solution. This solution is filtered and the filtrate is boiled for nearly two hours with 1·6 grms. of antimony trioxide. The filtrate on being concentrated and the side of the basin being scratched, a slightly yellow tinged substance separates. It is filtered and purified by crystallization twice from water. (Yield=2 grams.)

*This compound has been prepared with the idea of introducing antimony through the abraded skin or as an inunction.*

Calculated for $C_{12}H_{14}O_{10}$ NSb, Sb=26·54%. Found Sb=26·25%.

(6) *Preparation of 3 : 6 diamino-10-methyl acridinium antimonyl tartrate* (=*acriflavine antimonyl tartrate*)

0·65 grm. of 3 : 6 diamino-10-methyl acridinium chloride is dissolved in 20 c.c. of pyridine and the solution filtered. To the filtrate is added a solution of 1 grm. of silver antimonyl tartrate in 20 c.c. of pyridine. Reaction takes place at once and a light yellow precipitate is obtained. The whole mixture is then refluxed for at least four hours and the precipitate that is formed is collected by filtration. It is purified by washing thrice in hot pyridine, then several times with hot alcohol and subsequently with distilled water.

Calculated for $C_{18}H_{18}O_7SbN_3$, Sb=23·62%. Found Sb=23·92%. The compound is sparingly soluble in water and is lighter in colour and less fluorescent in solution than acriflavine.

*This compound was prepared with the idea of combining the therapeutic properties of antimony and acriflavine.*

## Remarks

The late Sir Patrick Manson once wrote to me as follows : "Go on in your efforts to get an antimony compound that can be used as an intramuscular injection or better still as a drug that can be administered by the mouth." It now requires to be seen how far the inference of the properties of the above antimonyl tartrates based on theoretical considerations is borne out in practice and how far they exert leishmanocidal properties. I shall enter into the subject in a subsequent series.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, March 13, 1923]

## PART VI

### CUMULATIVE AND TOLERANCE EXPERIMENTS WITH TARTAR EMETIC

THE following series of experiments have been made to determine whether repeated injections of tartar emetic into guinea-pigs in sub-lethal doses lead to tolerance towards the drug or its cumulative action.

| Serial No. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight. | Body weight in grms. at time of injection. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight. | Body weight in grms. at time of injection. |
|---|---|---|---|---|---|---|---|---|
| 771 ... | 1st | 25-8-22 | ·005 | 340 | 2nd | 28-8-22 | ·0075 | 355 |
| | 3rd | 31-8-22 | ·01 | 360 | 4th | 4-9 22 | ·015 | 355 |
| | 5th | 7-9-22 | ·02 | 360 | 6th | 12-9-22 | ·025 | 380 |
| | 7th | 18-9-22 | ·03 | 385 | 8th | 22-9-22 | ·035 | 360 |

*Remarks*—Animal died on 22nd September, 1922 with post-mortem signs of antimony poisoning. Total quantity injected—·1475 grm. per kilo.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 629 ... | 1st | 29-3-22 | ·018 | 355 | 2nd | 30-3-22 | ·018 | 340 |
| | 3rd | 31-3 22 | ·018 | 350 | 4th | 1-4-22 | ·018 | 355 |

*Remarks*.—Death on 1st April, 1922, showing post-mortem signs of antimony poisoning. Total amount injected—·072 grm. per kilo.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 564 ... | 1st | 6-4-22 | ·018 | 255 | 2nd | 7-4-22 | ·018 | 250 |
| | 3rd | 8-4-22 | ·018 | 255 | ... | ... | .. | ... |

*Remarks*.—Death on 10 April, 1922, showing post mortem signs of antimony poisoning. Total quantity injected—·054 grm. per kilo.

| Serial No. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight. | Body weight in grms. at time of injection. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight. | Body weight in grms. at time of injection. |
|---|---|---|---|---|---|---|---|---|
| 143 ... | 1st | 26-7-21 | ·0025 | 398 | 2nd | 8-8-21 | 0025 | 392 |
| | 3rd | 11-8-21 | ·003 | 412 | 4th | 19-8-21 | ·004 | 415 |
| | 5th | 24-8-21 | ·008 | 437 | 6th | 30-8-21 | ·01 | 435 |
| | 7th | 5-9-22 | ·01 | 450 | 8th | 15-9-21 | ·012 | 435 |
| | ... | 16-9-21 | ... | 415 | ... | 21-9-21 | ... | 425 |

*Remarks.*—Death on 22nd September, 1922, showing post-mortem signs of antimony poisoning. Total quantity injected—·052 per kilo.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 254 ... | 1st | 12-11-21 | ·005 | 195 | 2nd | 15-11-21 | ·005 | 196 |
| | 3rd | 19-11-21 | ·007 | 210 | 4th | 21-11-21 | ·007 | 200 |
| | 5th | 23-11-21 | ·008 | 210 | 6th | 30-11-21 | ·008 | 210 |
| | 7th | 4-12-21 | ·01 | 210 | 8th | 7-12-21 | ·01 | 210 |
| | 9th | 11-12-21 | ·015 | 230 | 10th | 13-12-21 | ·015 | 240 |
| | 11th | 16-12-21 | ·02 | 265 | 12th | 19-12-21 | ·02 | 260 |
| | ... | 20-12-21 | ... | 260 | 13th | 22-12-21 | .025 | 250 |
| | 14th | 25-12-21 | ·025 | 275 | 15th | 28-12-21 | ·03 | 262 |
| | 16th | 31-12-21 | ·04 | 280 | 17th | 3-1-22 | ·045 | 292 |
| | ... | 5-1-22 | ... | 285 | 18th | 8-1-22 | 05 | 295 |
| | 19th | 15-1-22 | ·06 | 320 | ... | 20-1-22 | ... | 322 |
| | 20th | 21-1-22 | ·07 | 330 | ... | ... | ... | ... |

*Remarks.*—Slightly restless just after injection on 11th December, 1921. Somewhat dull and averse to food after injection on 17th December, 1921. Somewhat dull and very much averse to food after injection on 20th December, 1921. Somewhat restless after injection on 22nd December, 1921. Restless after injection on 25th December, 1921. Restless after injection on 28th December, 1921. Animal became dull on 21st January, 1922, in the afternoon and died at night showing post-mortem signs of antimony poisoning. Total dose of tartar emetic injected per kilo of body weight—·475 grm.

| Serial No. | Injections. No. | Injections. Date | Dose in grms. per kilo of body weight | Body weight in grms. at time of injection. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight. | Body weight in grms. at time of injection. |
|---|---|---|---|---|---|---|---|---|
| 781 ... | 1st | 5-9-22 | ·0075 | 250 | 2nd | 8-9-22 | ·01 | 265 |
| | 3rd | 13-9-22 | ·015 | 280 | 4th | 18-9-22 | ·02 | 270 |
| | 5th | 22-9-22 | ·025 | 275 | 6th | 6-10-22 | ·03 | 265 |
| | 7th | 10-10-22 | 035 | 280 | ... | .. | ... | ... |

*Remarks.*—Animal became dull 6 hours after 5th injection. It began to lose weight. Animal died 10 hours after the last injection with post-mortem signs of antimony poisoning. Total quantity injected—·1425 grm. per kilo of body weight.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 540 ... | 1st | 27-3-22 | 01 | 300 | 2nd | 31-3-22 | 015 | 290 |
| | 3rd | 3-4-22 | ·018 | 305 | 4th | 7-4 22 | ·02 | 315 |
| | 5th | 12-4-22 | ·025 | 315 | 6th | 19-4 22 | ·03 | 330 |
| | 7th | 26-4-22 | ·04 | 330 | ... | ... | ... | ... |

*Remarks*—Somewhat restless after injections on 7th and 12th April, 1922. The animal began to lose weight considerably after injection on 19th April, 1922, but regained weight after 6 days. Restless after the injection on 26th April, 1922. The animal died 12 hours after injection showing post mortem signs of antimony poisoning. Total dose of tartar emetic injected per kilo of body weight—·158 grm.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 253 ... | 1st | 12-11-21 | ·005 | 215 | 2nd | 15-11-21 | ·005 | 217 |
| | 3rd | 19-11-21 | ·007 | 230 | 4th | 21-11-21 | ·007 | 220 |
| | 5th | 25-11-21 | ·008 | 230 | ... | ... | ... | ... |

*Remarks.*—Died on 25th November, 1921, showing post mortem signs of antimony poisoning. Total dose of tartar emetic injected per kilo of body weight—·032 grm.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 543 ... | 1st | 27-3-22 | ·01 | 325 | 2nd | 31-3-22 | 015 | 310 |
| | 3rd | 3-4-22 | ·018 | 330 | 4th | 7-4 22 | ·02 | 275 |

*Remarks*—Restless after injection on 7th April, 1922, and died the same night showing post-mortem signs of antimony poisoning. Total dose of tartar emetic injected per kilo of body weight—063 grm.

| Serial No. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight. | Body weight in grms. at time of injection. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight. | Body weight in grms. at time of injection. |
|---|---|---|---|---|---|---|---|---|
| 747 ... | 1st | 12-7-22 | ·005 | 370 | .. | 13 7-22 | ... | 360 |
| | 2nd | 16-7-22 | ·005 | 370 | ... | 17-7-22 | ... | 365 |
| | 3rd | 18 7-22 | ·01 | 365 | 4th | 20-7-22 | ·01 | 365 |
| | ... | 22 7-22 | ... | 362 | 5th | 24-7 22 | ·01 | 374 |
| | 6th | 27-7-22 | ·015 | 372 | 7th | 31 7-22 | ·02 | 365 |
| | 8th | 3-8-22 | ·025 | 360 | 9th | 6-8-22 | ·025 | 350 |
| | 10th | 9 8-22 | ·035 | 355 | 11th | 12-8 22 | ·04 | 356 |
| | 12th | 15 8-22 | ·045 | 360 | 13th | 20-8 22 | 05 | 370 |
| | 14th | 25-8-22 | 055 | 357 | 15th | 28-8-22 | ·055 | 312 |

*Remarks.*—Somewhat restless on 17th July, 1922. The animal remained dull for two days after injection on 20th August, 1922. The animal died on 28th August, 1922, 8 hours after injection. Total dcse of tartar emetic injected per kilo of body weight—·405 grm.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 748 ... | 1st | 15-7 22 | ·005 | 325 | 2nd | 18-7-22 | ·005 | 325 |
| | 3rd | 20-7-22 | ·0075 | 325 | 4th | 24-7-22 | ·01 | 342 |
| | ... | 29-7-22 | ... | 310 | 5th | 31-7-22 | ·01 | 315 |
| | 6th | 6-8-22 | 02 | 320 | ... | 7-8-22 | ... | 312 |
| | 7th | 9-8-22 | ·025 | 335 | ... | 10-8-22 | ... | 320 |
| | 8th | 12-8-22 | ·03 | 340 | 9th | 16-8-22 | ·035 | 340 |
| | ... | 18-8-22 | ... | 307 | 10th | 23-8-22 | ·04 | 320 |
| | 11th | 25-8-22 | ·0045 | 325 | 12th | 28-8-22 | ·05 | 325 |

*Remarks.*—Restless after injection on 24th July, 1922. Animal dull on 23rd August, 1922 Animal died on 28th August, 1922, showing post-mortem signs of antimony poisoning. Total dose of tartar emetic injected per kilo of body weight—·242 grm.

| Serial No. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight | Body weight in grms. at time of injection. | Injections. No. | Injections. Date. | Dose in grms. per kilo of body weight. | Body weight in grms. at time of injection. |
|---|---|---|---|---|---|---|---|---|
| 772 ... | 1st | 25-8-22 | ·005 | 402 | 2nd | 28-8-22 | ·0075 | 390 |
| | 3rd | 31-8-22 | ·01 | 410 | 4th | 5-9-22 | ·015 | 390 |
| | ... | 7-9-22 | ... | 375 | 5th | 8-9-22 | ·02 | 335 |
| | 6th | 13-9-22 | ·025 | 405 | 7th | 18-9-22 | ·03 | 405 |

*Remarks.*—Died on 19th September, 1922, showing post-mortem signs of antimony poisoning Total dose of tartar emetic injected per kilo of body weight – ·1125 grm.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 782 ... | 1st | 5-9-22 | ·0075 | 415 | 2nd | 8-9-22 | ·01 | 430 |
| | 3rd | 11-9-22 | ·015 | 425 | 4th | 18-9-22 | ·02 | 428 |
| | 5th | 22-9-22 | ·025 | 445 | ... | 23-9-22 | ... | 425 |
| | ... | 6-10-22 | ... | 430 | 6th | 10-10-22 | ·035 | 450 |
| | ... | 11-10-22 | ... | 435 | ... | 14-10-22 | ... | 430 |
| | 7th | 15-10-22 | ·04 | 440 | ... | 16-10-22 | ... | 430 |
| | ... | 18-10-22 | ... | 430 | ... | 19-10-22 | ... | 420 |
| | 8th | 20-10-22 | ·045 | 390 | 9th | 30-10-22 | ·05 | 390 |
| | 10th | 12-11-22 | ·055 | 365 | ... | ... | ... | ... |

*Remarks.*—The animal became dull 12 hours after injection on 22nd September, 1922 The animal became dull after injection on 15th October, 1922, and gradually lost weight since Death took place 8 hours after last injection. Total dose per kilo of body weight—·3025 grm.

## Conclusion

I have observed that repeated injections of tartar emetic in sub-lethal doses did not give rise to any tolerance towards the drug except very rarely. Generally the results pointed to a cumulative action of the drug, or at least made the animal susceptible to the next higher dose.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, March 13, 1923]

## PART VII

### DETERMINATION OF SMALL QUANTITIES OF ANTIMONY IN PRESENCE OF ORGANIC MATTER

The method followed by us in the quantitative determination of antimony in stools and urine depends upon the extraction of the antimony by boiling with copper and hydrochloric acid, and the subsequent solution of the antimony and its conversion into sulphide which is estimated colorimetrically. We at first tried the method described by Schidrowitz and Goldsbrough (*Analyst*, 1911, 36, 101) and its modification by Beam and Freak (*Analyst*, June, 1919). Both these methods gave unsatisfactory results in our hands, and the difficulties that we had to encounter were due to the presence of iron and the use of alkaline permanganate solution.

(1) In the process of extraction of antimony from urine, minute traces of iron are frequently deposited over the copper from the urine and go into solution in the subsequent treatment with alkaline permanganate solution. The iron seems to be converted into a soluble ferrate, from which it cannot be precipitated by the alkali in the alkaline permanganate solution. Beam and Freak's procedure does not remove this iron, which passes into the final test solution, giving rise, in the presence of antimony, to a reddish brown colour with

hydrogen sulphide which cannot be made to match with the yellow colour of pure antimonious sulphide solution.

(2) It seems that it is extremely difficult to prevent some manganese from passing into solution and its presence interferes considerably with the colorimetric test.

(3) As pointed out by Beam and Freak, the antimony precipitated on the copper becomes difficult to dissolve if it is not immediately treated with alkaline permanganate, and prolonged treatment with alkaline permanganate leads to the solution of copper.

We have therefore modified Beam and Freak's procedure to a considerable extent and our method is described as follows :—

(I) The whole of the twenty-four hours' urine is concentrated in a porcelain dish to about 50 c.c. by heating. The concentrated urine is then mixed with 10 c.c. of chemically pure concentrated hydrochloric acid and strips of pure copper foil of suitable size are introduced into the solution, which is boiled for some hours till all the antimony is deposited over the copper. To ensure complete precipitation of the antimony, a fresh strip of copper foil is introduced into the solution which is again boiled, after addition of a fresh quantity of chemically pure hydrochloric acid and distilled water, till no more black deposit forms on the copper. The strips of copper are then removed with glass forceps and, after washing in distilled water, are treated with an alkaline solution of persulphate of potassium.

(II) After the antimony has completely gone into solution, the latter is boiled with a slight excess of alkali to precipitate any traces of copper that may have gone into solution.

(III) The solution is filtered and sulphur dioxide passed into it for three to five minutes. The solution is boiled with hydrochloric acid which converts the iron into a chloride.

* (IV) After expelling the hydrochloric acid and the sulphur dioxide, the solution is boiled with potassium hydrate to precipitate the whole of the iron and is then filtered.

(V) The filtrate is acidified with hydrochloric acid and then a sufficient quantity of distilled water is added to make it up to 100 c.c.

(VI) To the solution 5 c.c. of 10 per cent gum solution is added and hydrogen sulphide passed through 50 c.c. of the solution until the colour is fully developed. Comparison is then made with a standard solution of pure tartar emetic treated in the same manner in Nessler's tubes and the amount of antimony thereby estimated.

To test the accuracy of the procedure the following estimations were made with chemically pure tartar emetic (sample supplied by Messrs. Martindale & Co., and certified by them to be one hundred per cent pure).

| Solution taken | | Antimony found |
|---|---|---|
| (1) ·0009 grm. of antimony | ... | ·00087 |
| (2) ·00144 ,, ,, ,, | ... | ·00141 |
| (3) ·0018 ,, ,, ,, | ... | ·00177 |
| (4) ·0027 ,, ,, ,, | ... | ·00267 |

In estimating antimony in the fæces, the stools are first mixed with water and boiled gently with hydrochloric acid for some hours and filtered. The fæces are then extracted with hot water several times till no more antimony can be detected in the filtrate. The whole of the filtrate is now treated in the same way as above to extract the total amount of antimony.

* If no iron has gone into solution, then (IV) is omitted.

## Remarks

(1) The use of alkaline persulphate avoids the introduction of manganese into the final test solution, the presence of which interferes with the colorimetric test to a considerable extent.

(2) Alkaline persulphate dissolves the deposit of antimony much more quickly than alkaline permanganate.

(3) It is essential to get rid of all traces of iron that may be precipitated from the urine on the copper foil and subsequently pass into solution.

(4) The method advocated here gives a fair degree of accuracy in estimating minute traces of antimony.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, April 14, 1923]

## PART VIII

### QUANTITATIVE STUDIES IN EXCRETION OF ANTIMONY (TARTAR EMETIC AND UREA STIBAMINE)

The excretion of antimony after administration of an antimonial to man or animals in the form of an inorganic or organic antimonial compound has received very little attention, though considerable attention has been given to the excretion of arsenic since the introduction of arseno-benzol compounds into therapeutics. Yet the former is just as important as the latter, since the discovery of the very important part played by antimony in therapeutics.

The antimonials that have been used for the determination of the rate of excretion of antimony consist of antimonyl tartrates and urea stibamine. Of the antimonyl tartrates, the rate of excretion of antimony after administration of tartar emetic will form the subject matter of the present paper. The antimonials studied here may be grouped together under the following heads: (1) Antimonials containing pentavalent antimony, e.g., urea stibamine, (2) antimonials containing trivalent antimony—the antimonyl tartrates. We did not study the rate of excretion of antimony after administration of stibacetin (Stibenyl), as the drug has not given satisfactory results in the treatment of kala-azar in the hands of many observers, and just as atoxyl or soamin has replaced ars-acetin in the treatment of diseases where one

intends to use pentavalent arsenic, so the salts of *p*-amino-phenyl-stibinic acid will perhaps replace stibacetin. (Antimonials of the stibinobenzene type have not yet come into use in the treatment of human diseases, though they have been used with indefinite results in the case of certain diseases of animals (*vide* Frankel's *Arzentimittel Synthese*).

By studying the excretion of antimony we have come to the conclusion that antimonials of the type of urea stibamine are converted within the body into trivalent oxide of antimony, similar to what happens in the case of organic pentavalent arsenicals, before they are capable of exhibiting organotropic and parasitotropic properties. This may be illustrated graphically in the following way :—

$$R.\,Sb\begin{cases} OH \\ = O \\ OR^1 \end{cases} \longrightarrow R.\,Sb = O.$$

It is possible that $-Sb=O$ is more reactive and more powerful in its parasiticidal properties when an organic antimonial is changed within the body into one containing it than when it exists in the antimonyl tartrates.

We may assume that the parasiticidal properties of antimony compounds depend upon the radicle $-Sb=O$, similar to what exists in the case of arsenicals, in which the parasiticidal properties depend upon $-As=O$."

In the present paper, our experimental work on the excretion of antimony is limited to observations in man. Though in some respects experiments on animals may be more convenient, yet the experiments conducted on man have the obvious advantage of giving an idea of the rate of excretion in man directly without the possible objection of having to deduce the same indirectly from observations on animals.

Our procedure was as follows : The individual, generally an adult, was asked to empty his bladder immediately before

injection and was injected intravenously generally between 9 A.M. and 10 A.M. daily. He was made to pass all his urine during 24 hours in perfectly clean bottles on the days of observation. The stools were similarly collected in perfectly clean vessels.

The same methods were adopted in the detection of antimony in the urine and the stools as were followed in its detection in the viscera (*see* Part III). The method adopted for the quantitative determination of antimony excreted has been described in the previous paper (*see* Part VII). As stated therein, the methods that have been described by previous workers for the quantitative estimation of antimony are misleading and we had to modify them considerably in order to obtain accurate results. It may be stated here that the estimation of antimony in the excreta tasks the patience of the experimenter to the utmost, requiring the most careful and delicate handling of all the processes. All the reagents and water used in the experiments must be previously tested to ensure that they are free from arsenic or antimony.

## Tartar Emetic

### *Period of Presence of Antimony in Urine*

### (*a*) After Single Injection

| | | | | Period during which antimony was present in the urine. |
|---|---|---|---|---|
| (1) | One dose of 2·5 c.c. of 2 per cent solution given intravenously | | ... | 1,560 hours. |
| (2) | Ditto | Ditto | Ditto ... | 774 ,, |
| (3) | Ditto | Ditto | Ditto ... | 1,108 ,, |
| (4) | Ditto | Ditto | Ditto ... | 1,060 ,, |

Average period—46 days.

## (*b*) After Repeated Injections

| | Period during which antimony was present in the urine. |
|---|---|
| (1) 42 c.c. of 2 per cent solution given in 15 injections (= ·84 grm.) | 1,152 hours after the last injection. |
| (2) 10 c.c. of 2 per cent solution in 5 injections (= ·2 grm ) ... | 1,192 hours after the last injection. |

Average period—49 days.

### *Daily Excretion of Antimony in Urine*

| (1) Injection of 2·5 c.c of 2 per cent solution (= ·018 Sb). | (2) Injection of 10 c.c. of 2 per cent solution (= ·072 Sb). | (3) Injection of 2·5 c c. of 2 per cent solution (= 0·18 Sb). | (4) Injection of 5 c.c. of 2 per cent solution (= ·036 Sb). |
|---|---|---|---|
| 1. ·00108 Sb | 1. ·003996 Sb | 1 ·001296 Sb | 1. ·002196 Sb |
| 2. ·00108 Sb | 2. ·003420 Sb | 2. ·00108 Sb | 2. ·00218 Sb |
| 3 ·00099 Sb | 3. ·002880 Sb | 3. ·00090 Sb | 3. ·001674 Sb |
| 4. ·00054 Sb | 4. 002562 Sb | 4. ·00063 Sb | 4. ·00117 Sb |
| 5. ·000324 Sb | 5. ·001800 Sb | 5. ·00045 Sb | 5. ·00108 Sb |
| 6. ·000324 Sb | 6. ·001480 Sb | 6. ·00035 Sb | 6. ·00108 Sb |
| | 7. 001170 Sb | | 7. ·00099 Sb |
| | 8. ·000972 Sb | | |
| | 9. ·000729 Sb | | |
| | 10. ·000648 Sb | | |
| | 11. ·000657 Sb | | |
| | 12. ·000648 Sb | | |

*1st Observation.*—

Quantity of Sb passed in 24 hours $= \frac{1}{16\cdot6}$ of the quantity injected.

Quantity of Sb passed in 6 days = ·004338 grm. or $\frac{1}{4\cdot1}$ oft he quantity injected.

*2nd Observation.*—

Quantity of antimony passed in 24 hours $= \frac{1}{18{\cdot}5}$ of the quantity injected.

Quantity of antimony passed in 6 days $= {\cdot}016138$ grm. or $\frac{1}{4{\cdot}5}$ of the quantity injected.

Quantity of antimony passed in 12 days $= {\cdot}020962$ grm. or $\frac{1}{3{\cdot}4}$ of the total quantity injected.

*3rd Observation.*—

Quantity of antimony passed in 24 hours $= \frac{1}{14}$ of the quantity injected.

Quantity of antimony passed in 6 days $= \frac{1}{3{\cdot}8}$ of the quantity injected.

*4th Observation.*—

Quantity of antimony psssed in 24 hours $= \frac{1}{16{\cdot}2}$ of the quantity injected.

Quantity of antimony passed in 6 days $= \frac{1}{3{\cdot}8}$ of the quantity injected.

## Urea Stibamine

*Period of Presence of Antimony in the Urine*

(*a*) After Single Injection

| | | | | | | Period during which antimony was present in the urine. |
|---|---|---|---|---|---|---|
| (1) | One dose of 2·5 c.c. of 2 per cent solution given intravenously | | | | ... | 744 hours |
| (2) | Ditto | Ditto | Ditto | Ditto | .. | 768 ,, |
| (3) | Ditto | Ditto | Ditto | Ditto | ... | 1,224 ,, |
| (4) | Ditto | Ditto | Ditto | Ditto | ... | 1,160 ,, |

Average - 40 days

## (b) After Repeated Injections

| | | | |
|---|---|---|---|
| (1) | 135 c.c. of 2 per cent solution given in 14 injections (2·7 grms.) | 1,536 | hours |
| (2) | 100 c.c. of 2 per cent solution given in 15 injections (2·0 grms.) | 768 | ,, |
| (3) | 97.5 c.c. of 2 per cent solution given in 15 injections (1·95 grms.) | 648 | ,, |

Average—41 days.

## *Daily Excretion of Antimony in Urine*

| 1 | 2 |
|---|---|
| Injection of 5 c.c. of a 2 per cent solution (= ·037 grm. Sb) | Injection of 5 c.c. of a 2 per cent solution (= ·037 grm). |
| 1. ·0149 grm. Sb | 1. ·01108 grm. Sb |
| 2. ·00189 grm. Sb | 2. ·0018 grm. Sb |
| 3. ·001 grm. Sb | 3. ·00126 grm. Sb |
| 4. ·0009 grm. Sb | 4. + 5. ·00144 grm. Sb |
| 5. ·0004 grm. Sb | |
| 6. ·00054 grm. Sb | 6. ·00072 grm. Sb |
| | 7. ·00036 grm. Sb |
| | 8. ·0003 grm. Sb |

*1st Observation.*—

Quantity of Sb passed in 24 hours = ·0149 Sb or $\frac{1}{2\cdot5}$ of total quantity injected.

Quantity of Sb passed in 6 days = ·01963 grm. Sb or $\frac{1}{1\cdot9}$ of the total quantity injected.

*2nd Observation.*—

Quantity of Sb passed in 24 hours = $\frac{1}{3\cdot34}$ of the quantity injected.

Quantity of Sb passed in 6 days = ·0163 grm. Sb or $\frac{1}{2\cdot24}$ of the total quantity injected.

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CHART I

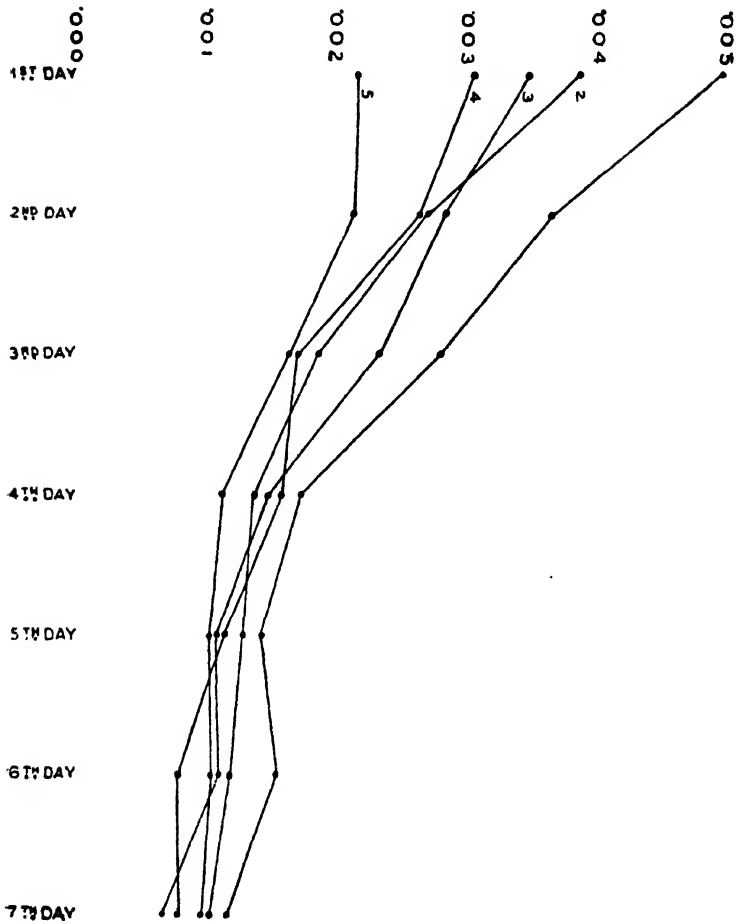
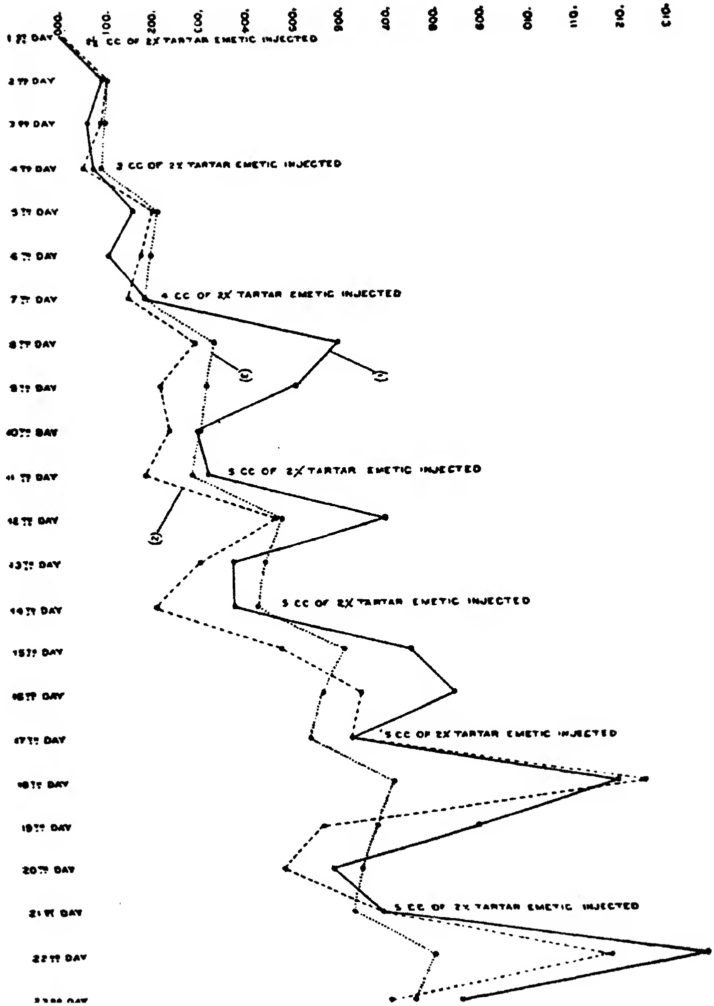



Chart showing the curve of excretion of antimonyl tartrates after intravenous injection in terms of antimony.

[*Reprinted from the Indian Journal of Medical Research, Vol. XI, No. 3, January, 1924*]

CHART II




1 and 2. **Curves of excretion of tartar emetic in terms of antimony after repeated injections of tartar emetic (2 cases).**

3. **A theoretical curve showing the excretion of tartar emetic in terms of antimony after repeated injections if the rate of excretion always followed the law that the amount excreted was proportional to the amount present in the system.**

We have observed that there is no relationship between the quantity of urine passed during a definite period and the excretion of antimony during the same period, as will be seen by the following figures :

*Curve of Excretion of Antimony in the Urine*

In the first observation, after injection with tartar emetic, the total quantity of urine passed in 24 hours was 590 c.c. and in 6 days it was 3,097 c.c. Amount of Sb passed in 24 hours = $\frac{1}{16{\cdot}6}$ and in 6 days $\frac{1}{4{\cdot}1}$ of the total quantity injected.

In the second observation, after injection with tartar emetic, the total quantity of urine passed in 24 hours was 1,625 c.c. and in 6 days it was 12,625 c.c. Amount of Sb passed in 24 hours was $\frac{1}{18{\cdot}5}$ and in 6 days was $\frac{1}{4{\cdot}5}$ of the total quantity injected. The quantity of urine passed by the second case was nearly three times the quantity passed by the first case during the first 24 hours. Similarly the quantity passed during the 6 days by the second case was four times that passed by the first case. The same phenomena were also observed in the case of urea stibamine.

Thus in the first observation, the amount of antimony passed in 24 hours was $\frac{1}{2{\cdot}5}$ and in 6 days $\frac{1}{1{\cdot}9}$ of the antimony injected. The amount of urine passed in 24 hours was 1,650 c.c. and the amount passed in 6 days was 8,225 c.c.

In the second observation the amount of antimony excreted in 24 hours was $\frac{1}{3{\cdot}34}$ and in 6 days it was $\frac{1}{2{\cdot}27}$ of the total quantity injected. The total quantity of urine passed

during 24 hours was 440 c.c., being nearly one-fourth the quantity passed in the first case. During 6 days, the amount passed was 2,665 c.c., being less than one-third the amount passed by the first case during the same period.

It will also be observed that in the case of tartar emetic the curve of excretion is one slowly converging to the base line, while in the case of urea stibamine the curve is abrupt during the first 24 hours and then follows the same course as in the case of tartar emetic.

It is evident that the excretion of antimony, after injection of urea stibamine, during the first 24 hours is quick and follows a similar course to what is observed in the case of organic pentavalent arsenicals, and then follows a curve like that in the case of tartar emetic which contains trivalent antimony. From this one may conclude that in the excretion of pentavalent antimonials, antimony is excreted as a pentavalent antimonial compound during the first 24 hours and then undergoes a reduction of the nature described before and in this process of excretion gives rise to a compound containing $-Sb=O$ in a reactive stage.

### *Excretion of Antimony in the Fæces and Vomit*

The excretion of antimony in the fæces is not so regular as that in the urine.

### Tartar Emetic

The excretion after intravenous injection of 10 c.c. of a 2 per cent solution of tartar emetic was as follows :—

(1) 1st 24 hours—no stool. (2) 2nd 24 hours—·001008 grm. Sb. (3) 3rd 24 hours—no stool. (4) 4th 24 hours—·000936 grm. Sb. (5) 5th 24 hours—no stool. (6) 6th 24

hours—·00072 grm. Sb. (7) 7th 24 hours—stool too small to be quantitatively determined.

Total quantity = ·002664 grm. Sb or $\frac{1}{27}$ of the total quantity injected.

After intravenous injection of 2·5 c.c. of a 2 per cent solution of tartar emetic, the amount of antimony present in the stools from the very beginning was too small to be quantitatively determined.

## Urea Stibamine

The excretion after injection of 5 c.c. of 2 per cent solution was as follows :

(1) 1st 24 hours—no stool. (2) 2nd 24 hours—·00072 grm. Sb. (3) 3rd 24 hours—·00054 grm. Sb. (4) 4th 24 hours—no stool. (5) 5th 24 hours—·00018 grm. Sb. (6) 6th 24 hours—too small to be quantitatively determined.

Total quantity = ·00144 grm. Sb or $\frac{1}{25}$ of the total quantity injected.

In another observation, the total quantity of antimony passed in the fæces after injection of 5 c.c. of 2 per cent solution was ·00063 grm. or less than $\frac{1}{60}$ of the total quantity injected.

In the vomit, immediately after the intravenous injection of tartar emetic, no antimony could be detected, confirming the observations of Weiss and Hatcher.

## Remarks

1. The amount of antimony excreted in the urine during the first 24 hours after intravenous injection of tartar emetic is about 6 per cent. of the amount injected.

2. The amount of antimony excreted in the urine during the first 24 hours after intravenous injection of urea stibamine is 30 to 40 per cent. of the amount injected.

3. The amount of antimony passed by the kidneys during the first 24 hours is fairly proportional to the amount injected.

4. There is no relationship between the quantity of urine passed during a certain period and the excretion of antimony during the same period. The effect of strong diuretics has not yet been studied.

5. The excretion of antimony after intravenous injection of tartar emetic follows a curve slowly converging to the base line.

6. The excretion of antimony after intravenous injection of a pentavalent organic antimonial follows a curve, the first portion of which, representing the excretion during the first 24 hours, is abrupt and the second portion follows a course similar to that found in the case of tartar emetic.

It is probable that a pentavalent organic antimonial is converted in the body into a trivalent antimonial and that as long as it exists in the body in the pentavalent form its rate of excretion is much quicker than when it is converted into the trivalent form. During the latter stage the curve of excretion is similar to that of tartar emetic in which antimony exists in the trivalent form.

7. After a single intravenous injection of an antimonial compound, the antimony may be present in the urine even for two months.

8. Since a great portion of antimony present in an aromatic pentavalent antimonial (urea stibamine) is quickly eliminated, the chances of toxic action of the compound are much less than that of an antimonyl tartrate.

9. In the process of conversion of an aromatic pentavalent antimonial in the body into a compound containing trivalent antimony, a reactive $-Sb=O$ is formed, which is

probably responsible for the remarkably beneficial results observed in the treatment of leishmaniasis by the use of urea stibamine.

10. The excretion of antimony by the fæces after intravenous injection of tartar emetic or a pentavalent antimonial is irregular and the quantity excreted is considerably less than in the urine.

11. The low toxicity of urea stibamine, the fact that no intolerance towards the drug is likely to take place on account of the quick elimination of a large proportion of it, the fact that it is perhaps converted in the body into a trivalent antimonial containing a reactive $-Sb=O$ radicle and the fact that its therapeutic value is very great (*vide Chemotherapy of Antimonial Compounds in Kala-azar Infection, Parts I and IV* in *Indian Journal of Medical Research*, October 1922, and 1923, and also Major Shortt's paper in the *Indian Medical Gazette*, July 1923, and in *Indian Journal of Medical Research*, October, 1923) make urea stibamine the best of all the antimonial compounds that have so far been discovered for the treatment of leishmaniasis and other diseases in which antimony is indicated.

The relation between excretion of antimony and renal functional activity, clinical character of the urine and physical and chemical characters of antimonial compounds used will be discussed in another series.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, September 17, 1923]

## PART IX

### TREATMENT OF CASES OF KALA-AZAR RESISTANT TO ANTIMONYL TARTRATES WITH UREA STIBAMINE—THE THERAPEUTIC VALUE OF STIBAMINE IN KALA-AZAR

(A)

The first series of kala-azar cases treated with urea stibamine appeared in the *Indian Journal of Medical Research* in October, 1922. The subsequent researches of Shortt have confirmed my observations and proved the remarkable leishmanicidal properties of the compound (*Indian Medical Gazette*, July, 1923). My further observations and those of Major Shortt on its use appeared in the *Indian Journal of Medical Research*, January, 1924. The following are Major Shortt's notes on his three recent cases cured by urea stibamine:

| | | |
|---|---|---|
| 1st case—Total urea stibamine given | | ·9 gramme in 5 injections. |
| 2nd case— | Do. | ·75 gramme in 4 injections. |
| 3rd case— | Do. | ·65 gramme in 4 injections. |

In each case the cure was established by subsequent observations in hospital and negative results obtained by culture of puncture material.

Writing to me recently on the use of this compound in kala-azar, Capt. C. Martin, I.M.S., of the Lawrence Military School, Sanawar, Simla Hills, stated as follows: "I am writing to tell you that the urea stibamine you sent me has worked wonders with one of my cases, which was not doing at all well with tartar emetic."

The compound is now having an extensive use in the wards of Lt.-Col. Barnardo, C.I.E., C.B.E., I.M.S., Principal, Medical College Hospitals, Bengal.

In the present paper is recorded the value of urea stibamine in a series of refractory cases of the disease:

By *refractory* or *resistant* cases of kala-azar, I mean cases which have resisted treatment with two grammes or more of sodium or potassium antimonyl tartrate given intravenously in the routine form of treatment of the disease extending over a period of two and a half months to three months or more. It is well known that a certain percentage of cases are not cured, or sometimes not even benefited by this dose of these antimonyl tartrates. Experience has also shown that all of these residual cases are not absolutely refractory, as some of them may be cured by a further course of treatment with antimonyl tartrates. Generally speaking, however, symptom of intolerance towards the drug appear in many cases after two grammes of the salt have been administered intravenously. Cases that have had this dose and show clinically little or no improvement and show the presence of leishmania in their tissues will be regarded as resistant or refractory for the purpose of this paper.

I have adopted this definition, as, some time ago, certain observations conducted in Shillong led to the conclusion that all cases of kala-azar were curable with 2 grammes

of tartar emetic. Subsequent observations did not confirm this view, as 34 out of 50 cases in Shortt's series were not cured with this dose. Out of these 34, 25 were subsequently cured by further treatment with sodium antimonyl tartrate for more or less prolonged periods, leaving a residual number of 9 which either died or left hospital uncured in spite of further treatment with sodium antimonyl tartrate. In Mackie's statistics, 13 out of 20 cases were not cured with this dose. Out of these 13, 4 were cured by further treatment with sodium antimonyl tartrate and Mackie considered that one was wholly refractory, the case being a boy of 10, showing living parasites after administration of 379 c.c. of one per cent solution of the salt, which would be equivalent to nearly 4 grammes of the antimonial. I shall, however, presently show that a case that resisted this dose was subsequently cured with a course of treatment with urea stibamine. In my own statistics in a series of 200 cases I have records of nearly 60 cases which resisted treatment with 2 grammes of sodium or potassium antimonyl tartrate or both combined, and 20 were not cured by 6 grammes of these salts. Generally speaking, it may be stated that cases that are not cured by 2 grammes of sodium or potassium salt require very prolonged treatment with these salts and even then, some may not be cured or benefited at all. It would be most interesting, if observers would publish records of failures in the treatment of kala-azar by the antimonyl tartrates, as many seem to believe that the last word about the treatment of the disease has been said in tartar emetic or sodium antimonyl tartrate. Unfortunately, this is not so. This, coupled with the fact that several cases require more than 4 or 5 months for a cure, demands the need for further advances in the present-day antimony treatment of kala-azar.

In the following series of refractory cases, the results obtained from the treatment with the antimonyl tartrates are

first described and the subsequent results obtained after intravenous injection of urea stibamine recorded.

(I) Patient named R.—History of fever for about six months. When he came to me for treatment his condition was as follows:

Fever—temperature 99° to 101°F. Spleen felt 6″ below the costal margin. Blood condition: R.B.C.—3,000,000, W.B.C.—3,500, Hb.—40 per cent. Peripheral blood culture and spleen puncture—positive.

Treatment with antimonyl tartrates: 6 grammes of sodium antimonyl tartrate and 2·2 grammes of potassium antimonyl tartrate twice a week extending over a period of nearly six months. Altogether 75 injections were given. Signs of intolerance began to show themselves after the 30th injection, consisting of vomiting, diarrhœa, severe cramps in the extremities at night during sleep and intense pain in the joints within 24 hours after injection. The treatment was still continued till another 45 injections were given.

During treatment patient was also given a course of soamin along with antimonyl tartrates for some time, 2 grammes of soamin having been given in 11 injections. To bring about leucocytosis he was given a trial with 6 injections of T.C.C.O., but to no effect.

The patient was then sent to Darjeeling, where he stayed for two months and a half but without any benefit.

Condition of the patient after treatment with antimonyl tartrates: Fever—still ranging between 99° to 101°F. Spleen felt 5″ below the costal margin. Blood condition: R.B.C.—3,250,000, W.B C.—4,500, Hb.—45 per cent. Peripheral blood culture and spleen puncture—positive. A refractory case.

Subsequent treatment with urea stibamine: The injections were given twice a week and the doses were (1) ·25 gramme, (2) ·2 gramme, (3) ·2 gramme, (4) ·25 gramme,

(5) ·25 gramme, (6) ·25 gramme, (7) ·25 gramme, (8) ·25 gramme, (9) ·25 gramme. Total quantity—2 grammes.

The patient began to improve after the second injection : the fever stopped after three injections and the condition of the patient was as follows, after the 9th injection :

Fever—no rise of temperature. Spleen could just be felt below the costal arch. Blood condition : R.B.C.—5,000,000, W.B.C.—6,250, Hb.—70 per cent. Peripheral blood and splenic blood culture and spleen puncture—negative.

The patient was under my observation for two months after his treatment was stopped, and when leaving Calcutta he was in excellent health. There was no enlargement of the spleen and the blood condition was : R.B.C.—5,000,000, W.B.C.—7,000, Hb.—75 per cent. Patient cured.

*N.B.*—The treatment with urea stibamine was started two months and a half after treatment with sodium antimonyl tartrate was discontinued during which all the antimony present in the patient's system must have been excreted, as I have shown in my paper *Chemotherapy of Antimonial Compounds in Kala-azar Infection, Part VIII* (*Indian Journal of Medical Research*, January, 1924) that the average period during which antimony is passed by the kidneys after repeated injections of sodium or potassium antimonyl tartrate is about a month and a half, and therefore the beneficial effects obtained subsequently in the patient could not be due to any residual antimony left in the patient's tissues after the previous treatment.

(II) Patient named Miss M.—History of fever for five months. When she came under my treatment, her condition was as follows : Fever—temperature ranging between 99° and 102°F. Spleen felt $3\frac{1}{2}''$ below the costal margin. Blood condition : R.B.C.—2,500,000, W.B.C.—3,000, Hb.—40 per cent. Peripheral blood culture and spleen puncture—positive.

Treatment with sodium antimonyl tartrate : 4·6 grammes of sodium antimonyl tartrate in 55 injections given twice a week extending over a period of nine months. Signs of intolerance began to show themselves after the 25th injection, consisting of palpitation and high fever followed by heavy sweats. The treatment was continued till another 30 injections were given.

During the course of treatment, there was some improvement in the condition of the patient and the fever stopped for some time, but recurred again. The condition of the patient after the course of treatment with sodium antimonyl tartrate was as follows : Fever—temperature irregular with periods of apyrexia from time to time. Spleen felt $2\frac{1}{2}''$ below the costal margin. Blood condition : R.B.C.—3,000,000, W.B.C.—4,000, Hb.—45 per cent. Peripheral blood culture and spleen puncture—positive. A refractory case.

Subsequent treatment with urea stibamine : Altogether 8 injections were given in doses of ·2 gramme each, twice a week. The patient began to improve after the third injection, there being no rise of temperature and the spleen diminished in size quickly. The condition of the patient one month after cessation of treatment was as follows : Fever—no rise of temperature for nearly two months. Spleen slightly felt below the costal arch. Blood condition : R.B.C.—4,000,000, W.B.C.—7,500, Hb.—65 per cent. Peripheral and splenic blood culture and spleen puncture—negative. The patient was examined by me again six months after completion of treatment and she was in excellent health. Patient cured.

(III) Patient J., æt. 24.—The following is the history of his case :

(1) He had two courses of treatment in Dacca with intravenous injection of tartar emetic at an interval of two months between them. During the first course he had 1·2 grammes in 14 injections and in the second ·8 gramme in

12 injections. He left the treatment without much benefit, there being no diminution in the height of the fever and in the size of the spleen.

(2) He was admitted into the Berry White Hospital in Dibrugarh after a fortnight. His condition on admission was as follows : Temperature—a double rise ranging between 99° and 103°F. R.B.C.—2,420,000, W.B.C.—2,810, Hb.—50 per cent. Spleen enlarged, extending 3″ below the costal arch. Spleen puncture—positive.

Patient was given here a course of treatment with sodium antimony tartrate from 16th November, 1920 to 4th April, 1921, the total dose being 2 grammes. Towards the latter part of the treatment, symptoms of intolerance began to show themselves, such as vomiting, harassing cough and pain in the joints. The last injection was followed by a temporary collapse. Thare was no improvement in his temperature and the spleen was felt 3″ below the costal arch. There was an improvement in the blood condition; the leucocytes numbered 6,000 per c.m., but otherwise the condition of the patient was not improved.

(3) He was admitted into my ward on 7th January, 1922, six months after the treatment was stopped. His condition was as follows : Temperature—varying between 99° to 101°F. Spleen 7″ below the costal arch. R.B.C.—2,500,000, W.B.C.—2,400, Hb.—30 per cent. Spleen puncture—positive.

The patient was given intravenous injections of tartar emetic twice a week, extending over a period of nearly four months. Altogether 30 injections were given (=3·75 grammes). He was discharged on 18th May, 1923. His condition at the time of discharge was as follows : Freedom from fever after the 18th injection. Spleen 3½′ below the costal margin. R.B.C.—3,600,000, W.B.C.—4,000, Hb.—52 per cent. Spleen blood culture was positive.

(4) After a month and a half, he was again admitted into the Campbell Hospital in a miserable state. L. D. bodies were found on spleen puncture and the leucocyte count was 2,400, the spleen extending 5″ below the costal arch. He was again treated with intravenous injection of sodium and potassium antimonyl tartrate given alternately, altogether 2·5 grammes being administered in 30 injections. There was no improvement in the patient's condition. He was kept under observation for nearly two months after which his condition was as follows: Spleen 4″ below the costal arch. Temperature ranging between 99° and 100°F. R.B.C.—2,200,000, W.B.C.—3,200, Hb.—48 per cent. Spleen puncture positive. A refractory case.

(5) Subsequent treatment with urea stibamine: 3·5 grammes of urea stibamine were given in 15 injections, twice a week.

Effect of treatment: Two months after commencement of treatment. Temperature—no rise of temperature for nearly a month. R.B.C.—4,500,000, W.B.C.—8,400, Hb.—60 per cent. Spleen could just be felt below the costal arch. Peripheral and splenic blood culture and spleen puncture—negative. Increase of body weight by one stone.

Six months after completion of treatment, the patient's condition was as follows: Increase of body weight by one stone. Temperature—no rise of temperature since leaving hospital. R.B.C.—4,200,000, W.B.C.—9,000, Hb.—62 per cent. Peripheral blood culture—negative. Spleen not felt below the costal arch. Patient cured.

*N.B.*—In this case too, the treatment with urea stibamine was started when all the antimony from the previous treatment was eliminated from the tissues, during the two months when the patient had no antimony treatment.

(IV) Patient Mr. L.—Condition on admission: Spleen 7″ below the costal arch. Temperature—99° to

100°F. Peripheral blood culture and spleen puncture—positive. R.B.C.—3,000,000, W.B.C.—2,400, Hb.—50 per cent.

Treatment with tartar emetic : 2·8 grammes given in 40 injections, extending over a period of six months. Effect of treatment—general condition worse than before, with loss of weight and fever of a low intermittent type. Peripheral blood culture and spleen puncture—positive. Spleen 6″ below the costal arch. A resistant case.

Subsequent treatment with urea stibamine commenced one month after treatment with tartar emetic was stopped, 1·6 grammes in 9 injections in doses of ·1 to ·25 gramme being given during one month and a half. Effect of treatment—one month after completion of treatment, general condition improved. Increase of body weight by half stone. Spleen not felt below the costal arch. Peripheral blood culture—negative. Fever stopped after administration of ·5 gramme of urea stibamine. Two months after completion of treatment, blood condition was as follows: R.B.C.—4,900,000, W.B.C.—7,800, Hb.—60 per cent. There was increase of body weight by one stone. Patient cured.

(V) Patient R.—Patient was admitted into hospital after he had 25 injections of 2 per cent solution of sodium antimonyl tartrate and potassium antimonyl tartrate given alternately over a period of three months. Total quantity—1 gramme.

Condition on admission : Temperature 99° to 100°F. Spleen 8″ below the costal arch. R.B.C —2,600,000, W.B.C.—1,200, Hb.—40 per cent. Peripheral blood culture and spleen puncture—positive. Patient was suffering from dysentery.

Treatment with sodium antimonyl tartrate : 4 grammes in 40 injections over a period of four months, given mostly on alternate days.

Effect of treatment : Temperature—no effect. Spleen same as before. R.B.C. –2,800,000, W.B.C.—3,200, Hb.—38 per cent. Peripheral blood culture and spleen puncture—positive. A refractory case.

Subsequent treatment with urea stibamine : 3 grammes given in 12 injections on alternate days during a period of one month.

Effect of treatment : Two and a half months after completion of treatment, no fever for three months. Spleen could not be felt below the costal arch. R.B.C.—4,600,000, W.B.C.—7,200, Hb.—62 per cent. Peripheral blood culture—negative. Increase of body weight by one stone. Patient cured.

(VI) Patient M.—Patient was admitted into hospital after he had taken 8 intravenous injections of sodium antimonyl tartrate. Condition: Speen 7″ below the costal arch. Temperature 99° to 101°F. R.B.C.—2,300,000, W.B.C.—1,000, Hb.—40 per cent. Peripheral blood culture and spleen puncture—positive.

Treatment with sodium antimonyl tartrate : 5 grammes injected in 40 injections over a period of five months.

Result of blood examination : Spleen 5″ below the costal arch. Fever—slight improvement. R.B.C.—3,800,000, W.B.C.—3,600, Hb.—48 per cent. Peripheral and splenic blood culture—positive. A refractory case with slight improvement.

Subsequent treatment with urea stibamine: 2·2 grammes in 9 injections given over a period of two months.

Effect of treatment: Spleen 2½″ below the costal margin. Freedom from fever for a month and a half. R.B.C.—4,800,000, W.B.C.—6,800, Hb.—60 per cent. Peripheral and splenic blood culture—negative. Spleen puncture—negative. Five months after completion of treatment spleen could not be felt below the costal arch.

Increase of body weight by one stone. Peripheral blood culture—negative. R.B.C.—4,800,000, W.B.C.—8,000, Hb.—62 per cent. Patient cured.

(VII) Patient Sarogoo. Condition on admission: Temperature—99° to 103°F. Spleen 6″ below the costal arch. R.B.C.—2,600,000, W.B.C.—2,800, Hb.—40 per cent. Peripheral blood culture and spleen puncture—positive.

Treatment with sodium antimonyl tartrate: 3 grammes injected in 28 injections extending over a period of four months.

Effect of treatment: Temperature—no effect. Spleen same as before. Decrease of body weight by one stone. R B.C.—3,600,000, W.B.C.—3,000, Hb.—40 per cent. Peripheral blood culture and spleen puncture—positive. A resistant case.

Subsequent treatment with urea stibamine: 2·4 grammes injected in 10 injections extending over a period of a month and a half.

Effect of treatment: Freedom from fever 14 days after commencement of treatment. Two months after completion of treatment, spleen not felt below the costal arch. Increase of body weight by one stone. R.B.C.—4,300,000, W.B.C. —7,000, Hb.—60 per cent. Peripheral blood culture—negative after 10th injection. Patient cured.

(VIII) Patent N., æt 36, was admitted into my ward in the Campbell Hospital with *cancrum oris* and œdema of the extremities.

Condition on admission: Spleen 5½″ below the costal arch. Peripheral blood culture and spleen puncture—positive. R.B.C.—2,400,000, W.B.C.—1,000, Hb.—26 per cent. Temperature—100° to 103°F.

Treatment with sodium antimonyl tartrate: 3·8 grammes given intravenously in 45 injections extending over a period of six months. Effect of treatment: There was no improvement in the general condition, no diminution in the size of

the spleen, œdema marked, loss of weight by 10 lbs., *cancrum oris*—diminished. R.B.C.—3,200,000, W.B.C.—2,500, Hb.—32 per cent. Peripheral blood and spleen blood culture—positive. Temperature—99° to 101°F. Symptoms of intolerance appeared after injection of 2·8 grammes of sodium antimonyl tartrate. A refractory case.

Subsequent treatment with urea stibamine: 2·8 grammes given in 13 injections, extending over a period of two months in doses of ·1 to ·25 gramme.

Effect of treatment: General condition—great improvement. Increase of body weight by one stone. Spleen not felt below the costal arch. R.B.C.—4,100,000, W.B.C.—6,200, Hb.—54 per cent. Peripheral blood culture—negative. Patient cured.

(IX) Patient B., æt. 30, was admitted into my ward in the Campbell Hospital with fever of six months' duration and œdema of the extremities.

Condition on admission: Spleen 7″ below the costal arch. Spleen puncture and peripheral blood culture—Positive. R.B.C.—2,500,000, W.B.C.—2,400, Hb.—30 per cent. Temperature—99° to 102°F.

Treatment with sodium antimonyl tartrate: 3·35 grammes of sodium antimonyl tartrate given in 35 injections, extending over a period of four months.

Effect of treatment: Fever came down aftar the 15th injection, spleen not reduced in size, emaciation with marked œdema. R.B.C.—2,200,000, W.B.C.—1,000, Hb.—25 per cent. Spleen puncture—positive. Peripheral and splenic blood culture—positive. A refractory case.

Subsequent treatment with urea stibamine: 2·2 grammes given in 12 injections, extending over a period of a month and a half.

Effect of treatment: General condition improved, increase of body weight by 9 lbs., peripheral and splenic blood culture—negative. R.B.C.—3,600,000, W.B.C.—7,000,

Hb.—60 per cent. Spleen just felt below the costal arch on deep inspiration. Patient cured.

A further series of six cases that have resisted treatment with more than four grammes of tartar emetic or sodium antimonyl tartrate are rapidly improving under urea stibamine.

Purity of the antimonyl tartrates used: The sodium and potassium antimonyl tartrates used in the above cases were chemically pure and especially made for me for purpose of research.

### *Possibility of Using Urea Stibamine Intramuscularly*

Both Major Shortt and myself are using the compound intramuscularly. The local reaction is generally slight. It is possible that the compound may be advantageously used intramuscularly. A report on this subject will be communicated later on.

Urea stibamine, being prepared under perfectly aseptic conditions, is sterile and has been found to be so by being repeatedly tested on culture media. Its solution should not be boiled. The same precautions should be taken in making its solution as in the case of neo-salvarsan.

## REMARKS

It will be seen that the duration of treatment and the number of injections required for complete cure of the cases recorded in the present paper were less in this series than in two former series already reported by me (*Indian Journal of Medical Research*, October, 1922 and October, 1923). This confirms the observations of Major Shortt who also found that the course of treatment required in his cases was much less than what was apparently required in my first two series. In other words, sterilization in these cases must have taken place earlier than I thought and I must

have continued my treatment for a longer period than was really necessary for a complete cure.

## (B)

### *Therapeutic Value of Stibamine*

Stibamine is the name given by me to *p*-amino-phenyl stibinate of sodium (*Journal of Tropical Medicine and Hygiene*, 15th August, 1921). It bears the same relation to stibacetin as atoxyl or soamin does to ars-acetin. Its chemical and physical properties have already been described and its toxicity tested by me (*Indian Journal of Medical Research*, October, 1922). Unless perfectly neutral, its solution in water is likely to decompose. The pure salt is fairly stable. In the *Indian Medical Gazette*, January, 1923, it has been stated by Chopra and Napier that the compound was very easily decomposed. Evidently, the substance that they were using was impure.

### *Cases Treated with Stibamine*

(I) Patient Rishi Kesh, æt. 30. History of fever—about a year. Admitted into my ward at the Campbell Hospital, on 25th August, 1922. Condition on admission: anæmic, spleen 5″ below the costal arch. Temperature—100° to 103°F. R.B.C.—2,600,000, W.B.C.—2,600, Hb.—40 per cent. Peripheral blood culture and spleen puncture—positive.

Treatment with stibamine: 2·4 grammes were given in 20 injections intravenously in the course of 54 days. Blood culture was positive 26 days after commencement of treatment, when 10 injections were given. After completion of treatment, the patient improved in weight by 1 stone 2 lbs. Spleen could just be felt below the costal arch. R.B.C.—5,100,000, W.B.C.—8,000, Hb.—62 per cent. Peripheral

and spleen blood culture—negative. Spleen puncture—negative. Fever stopped after 8 injections. Patient cured.

(II) Patient Kali, æt. 12. History of fever—six months. Admitted into my ward at the Campbell Hospital on 22nd August, 1922. Condition on admission : Spleen 3½″ below the costal arch. Temperature—99° to 101°F. R. B. C.—3,600,000, W.B.C.—2,400. Hb.—50 per cent. Peripheral and spleen blood culture—positive. Spleen puncture—positive.

Treatment with stibamine : 1·2 grammes were given in 18 injections intravenously in course of 48 days. Blood culture was positive 24 days after commencement of treatment during which ·8 gramme was given in 12 injections. After completion of treatment the patient increased in weight by 1 stone 8 lbs. Spleen could not be felt below the costal arch. R.B.C.—4,200,000, W.B.C.—6,000, Hb.—54 per cent. Peripheral blood culture—negative. Two months after completion of treatment, blood culture—negative. The patient was under my observation eight months after commencement of treatment. Patient cured.

(III) Patient D'Cruz, æt. 35 years. History of fever—a year and a half. Admitted into my ward at the Campbell Hospital on 20th August, 1922.

Condition on admission : R.B.C.—3,100,000, W.B.C.—3,600, Hb.—40 per cent. Peripheral and spleen blood culture—positive. Spleen puncture—positive. Spleen 7″ below the costal arch. Temperature—varying from 98° to 99·6°F.

Treatment with stibamine : 4·4 grammes in 30 injections intravenously over a period of 100 days. After the treatment, patient improved in weight by 11 lbs. Fever stopped after 18 injections. Peripheral and spleen blood culture—negative. Spleen puncture—negative. R.B.C.—3,900,000, W.B.C.—6,800, Hb.—50 per cent. The spleen still remained enlarged extending 5″ below the costal arch. Three months after completion of treatment spleen was 2½″ below

costal arch. No leishmania found on culture of the splenic and peripheral blood. Spleen puncture—negative. No fever since patient left hospital. Patient cured.

## Remarks

The above three cases show the curative value of stibamine. No attempt will be made in the present paper to give a comparative estimate of the therapeutic values of stibamine and urea stibamine. Its toxicity is slightly greater than that of urea stibamine (*Indian Journal of Medical Research*, October, 1922).

## Observations

(1) The curative value of urea stibamine and its superiority over the antimonyl tartrates have been established by research conducted by me as well as by Major Shortt in different places.

(2) Refractory cases that resisted treatment with antimonyl tartrates have yielded to urea stibamine.

(3) The short course of treatment and the lesser number of injections required in bringing about a complete cure are striking. Up to now, no case of kala-azar has been met with, which has been resistant to urea stibamine.

(4) No relapse has been met with among the cases that have undergone complete treatment with urea stibamine; some of these have been under my observation for nearly two years after completion of treatment.

(5) No relapse has up to now been met with among the resistant cases that have subsequently been cured by urea stibamine. Some of these cases have been under my observation for about a year.

(6) The therapeutic value of stibamine is recorded in three cases.

(7) The possibility of using urea stibamine intramuscularly is suggested.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, February 14, 1924]

## PART X

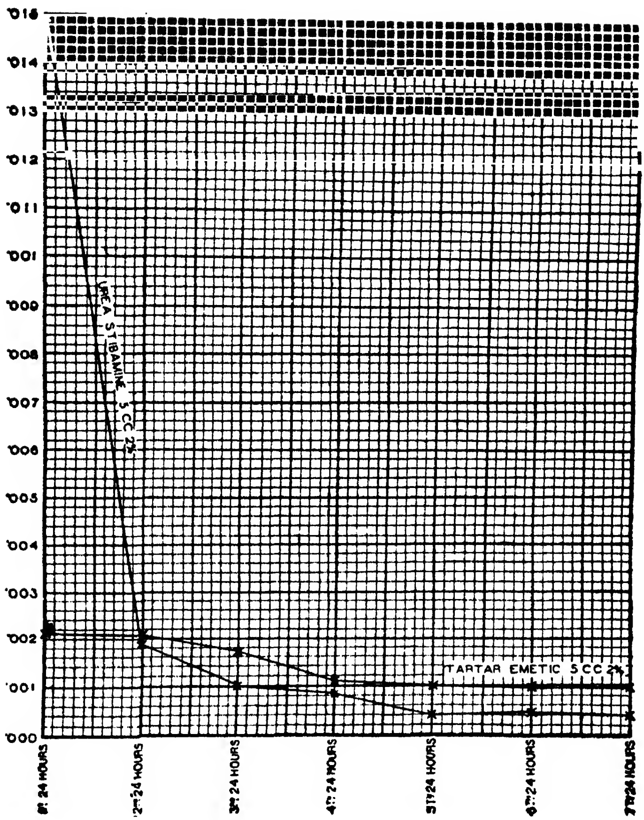
### FURTHER OBSERVATIONS ON QUANTITATIVE STUDIES IN EXCRETION OF ANTIMONY—THE INFLUENCE OF THE BASIC RADICLE AND OF REPEATED INJECTIONS OF AN ANTIMONYL TARTRATE UPON THE EXCRETION OF ANTIMONY

### (A)

### THE INFLUENCE OF THE BASIC RADICLE OF AN ANTIMONYL TARTRATE UPON THE EXCRETION OF ANTIMONY

In our last paper on quantitative studies in excretion of antimony, we discussed the excretion of antimony after intravenous injection of tartar emetic. Further observations have since been made to determine the excretion of antimony after injection of the different antimonyl tartrates. In the present paper we shall make a comparative quantitative study of antimony excretion after injection of the following tartrates :

(1) Tartar emetic.
(2) Sodium antimonyl tartrate.
(3) Lithium antimonyl tartrate.
(4) Ammonium antimonyl tartrate.
(5) Urea antimonyl tartrate.




Excretion of antimony after injection of tartar emetic and urea stibamine.

## (1) Tartar Emetic

The following are summarised from our previous paper on the excretion of antimony after injection of tartar emetic.

(*a*) Period of presence of antimony in the urine after a single injection of tartar emetic = 46 days on the average.

(*b*) The same after repeated injections = 49 days on the average after the last injection.

(*c*) Total quantity of antimony excreted in 24 hours :—

1st observation $\frac{1}{16 \cdot 6}$ of the total quantity injected.

2nd observation $\frac{1}{18 \cdot 5}$ of the total quantity injected.

3rd observation $\frac{1}{14}$ of the total quantity injected.

Total quantity excreted in seven days :—

1st observation $\frac{1}{4 \cdot 3}$ of the total quantity injected.

2nd observation $\frac{1}{3 \cdot 4}$ of the total quantity injected.

## (2) Sodium Antimonyl Tartrate

(I) Period of presence of antimony in the urine after a single injection :—

| | | | |
|---|---|---|---|
| 1st observation after injection of 2·5 c.c. of a 2 per cent. solution | | | 1,200 hours. |
| 2nd observation | ,, | ,, | 1,560 ,, |
| 3rd observation | ,, | ,, | 916 ,, |

Average = 51 days.

(II) Period of presence of antimony in the urine after repeated injections of sodium antimonyl tartrate :—

1st observation.—9 c.c. of a 2 per cent solution given in 4 injections—1,380 hours after last injection.

2nd observation.—29 c.c. of a 2 per cent solution given in 13 injections—1,584 hours.

3rd observation.—32 c.c. of a 2 per cent solution given in 15 injections—1,056 hours.

4th observation.—30 c.c. of a 2 per cent solution given in 14 injections—1,042 hours.

Average period = 52 days.

*Daily Excretion of Antimony in the Urine*

1st observation.—Injection of 5 c.c. of a 2 per cent solution (= ·0341 grm. Sb).

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 225 c.c. | ·00288 grm. |
| 2 | 375 ,, | ·002826 ,, |
| 3 | 525 ,, | ·0018 ,, |
| 4 | 720 ,, | ·00171 ,, |
| 5 | 940 ,, | ·00117 ,, |
| 6 | 975 ,, | ·00099 ,, |
| 7 | 975 ,, | ·000936 ,, |

Total quantity of antimony passed in 24 hours = $\frac{1}{11 \cdot 8}$ of the quantity injected.

Total quantity passed in seven days = ·012312 grm. or $\frac{1}{2 \cdot 77}$ of the total quantity injected.

2nd observation.—Injection of 5 c.c. of a 2 per cent solution (= ·0341 grm. Sb).

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 1,250 c.c. | ·00324 grm. |
| 2 | 285 ,, | ·002464 ,, |
| 3 | 1,000 ,, | ·0014 ,, |
| 4 | 1,450 ,, | ·0017 ,, |
| 5 | ... | ·00115 ,, |
| 6 | 1,100 ,, | ·0006 ,, |
| 7 | 1,625 ,, | ·00068 ,, |

Total quantity of antimony passed in 24 hours = $\frac{1}{10 \cdot 5}$ of the total quantity injected.

Total quantity passed in seven days = ·011234 grm. or $\frac{1}{3 \cdot 03}$ of the total quantity injected.

### (3) Lithium Antimonyl Tartrate

Period of presence of antimony after a single injection of 5 c.c. of a 2 per cent solution of lithium antimonyl tartrate.

1st observation.—478 hours (5 c.c. of a 2 per cent solution injected).
2nd observation.—456 hours ditto
3rd observation.—312 hours ditto

Average period = 17 days.

*Daily Excretion of Antimony in the Urine*

1st observation.—Injection of 5 c.c. of a 2 per cent solution (= ·0406 grm. Sb).

| Day. | Amount of urine passed | Amount of antimony passed. |
|---|---|---|
| 1 | 1,750 c.c. | ·006 grm. |
| 2 | 1,900 ,, | ·0028 ,, |
| 3 | ... | ·0021 ,, |
| 4 | 2,000 ,, | ·0014 ,, |
| 5 | 1,200 ,, | ·00162 ,, |
| 6 | 1,300 ,, | ·00162 ,, |
| 7 | 1,250 ,, | ·00108 ,, |

Total quantity of antimony passed in 24 hours $= \frac{1}{6{\cdot}76}$ of the quantity injected.

Total quantity passed in seven days = ·01662 grm. or $\frac{1}{2{\cdot}44}$ of the total quantity injected.

2nd observation.—Injection of 5 c.c. of a 2 per cent solution (= ·0406 grm. Sb).

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 1,000 c.c. | ·00445 grm. |
| 2 | 500 ,, | ·00405 ,, |
| 3 | 320 ,, | ·002754 ,, |
| 4 | 275 ,, | ·00162 ,, |
| 5 | 425 ,, | ·00126 ,, |
| 6 | 650 ,, | ·0014 ,, |
| 7 | 550 ,, | ·000864 ,, |

Total quantity of antimony passed in 24 hours $= \frac{1}{9{\cdot}1}$ of the quantity injected.

Total quantity passed in seven days = ·016498 grm. or $\frac{1}{2{\cdot}46}$ of the total quantity injected.

3rd observation.—Injection of 5 c.c. of a 2 per cent solution (= ·0406 grm. Sb).

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 1,095 c.c. | ·0044 grm. |
| 2 | 950 ,, | ·004100 ,, |
| 3 | 830 ,, | ·003600 ,, |
| 4 | 2,300 ,, | ·002146 ,, |
| 5 | 2,560 ,, | ·001890 ,, |
| 6 | 2,650 ,, | ·001700 ,, |
| 7 | 2,175 ,, | ·001379 ,, |

Total quantity of antimony passed in 24 hours = $\frac{1}{9 \cdot 2}$ of the quantity injected.

Total quantity passed in seven days = ·019215 grm. or $\frac{1}{2 \cdot 1}$ of the total quantity injected.

### (4) Ammonium Antimonyl Tartrate

(I) Period of presence of antimony in the urine after a single injection of 5 c.c. of a 2 per cent. solution of ammonium antimonyl tartrate :—

| | |
|---|---|
| 1st observation ... ... ... | 600 hours. |
| 2nd observation ... ... ... | 576 ,, |
| 3rd observation ... ... ... | 504 ,, |
| 4th observation ... ... ... | 480 ,, |

Average period—23 days approximately.

(II) Period of presence of antimony in the urine after repeated injections :—

7 injections ... ... 888 hours or 37 days.

*Daily Excretion of Antimony in the Urine*

1st observation.—Injection of 5 c.c. of a 2 per cent solution of ammonium antimonyl tartrate (= ·0381 grm. Sb) .

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 1,650 c.c. | ·003564 grm. |
| 2 | 1,750 ,, | ·00288 ,, |
| 3 | 2,045 ,, | ·00279 ,, |
| 4 | 2,350 ,, | ·00162 ,, |
| 5 | 1,075 ,, | ·001044 ,, |
| 6 | 3,500 ,, | ·00135 ,, |
| 7 | 1,775 ,, | ·000527 ,, |

Total quantity of antimony passed in 24 hours = $\frac{1}{10\cdot7}$ of the quantity injected.

Total quantity passed in seven days = ·013775 grm. or $\frac{1}{2\cdot76}$ of the quantity injected.

2nd observation.—Injection of 5 c.c. of a 2 per cent solution of ammonium antimonyl tartrate (= ·0381 grm. Sb).

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 715 c.c. | ·00315 grm. |
| 2 | 575 ,, | ·002128 ,, |
| 3 | 675 ,, | ·00206 ,, |
| 4 | 320 ,, | ·00161 ,, |
| 5 | 360 ,, | ·00129 ,, |
| 6 | 500 ,, | ·0012 ,, |
| 7 | 1,375 ,, | ·0009 ,, |

Total quantity of antimony passed in 24 hours = $\frac{1}{12\cdot1}$ of the quantity injected.

Total quantity passed in seven days = ·012338 grm. or $\frac{1}{3\cdot1}$ of the quantity injected.

3rd observation.—Injection of 5 c.c. of a 2 per cent solution of ammonium antimonyl tartrate (= ·0381 grm. Sb).

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 1785 c.c. | ·00378 grm. |
| 2 | 1450 ,, | ·0036 , |
| 3 | 1320 ,, | ·00229 ,, |
| 4 | 1180 ,, | ·00129 ,, |
| 5 | 1760 ,, | ·001 ,, |
| 6 | ... | ·000788 ,, |
| 7 | 1230 ,, | ·000576 ,, |

Total quantity of antimony passed in 24 hours = $\frac{1}{10 \cdot 1}$ of the quantity injected.

Total quantity of antimony passed in seven days = ·013324 grm. or $\frac{1}{2 \cdot 86}$ of the total quantity injected.

## (5) Urea Antimonyl Tartrate

Period of presence of antimony in the urine after a single injection of 5 c.c. of a 2 per cent solution :—

| | |
|---|---|
| 1st observation ... ... | 456 hours. |
| 2nd observation ... ... | 456 ,, |
| 3rd observation ... ... | 384 ,, |

Average = 18 days.

*Daily Excretion of Antimony in the Urine*

1st observation.—Injection of 5 c.c. of a 2 per cent solution of urea antimonyl tartrate (= ·03175 grm. Sb).

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 900 c.c. | ·0036 grm. |
| 2 | 1,000 ,, | ·00378 ,, |
| 3 | 780 ,, | ·00165 ,, |
| 4 | 1,200 ,, | ·00144 ,, |
| 5 | 700 ,, | ·0014 ,, |
| 6 | 1,030 ,, | ·001242 ,, |
| 7 | 1,030 ,, | ·001 ,, |

Total quantity of antimony passed in 24 hours = $\frac{1}{8 \cdot 8}$ of the quantity injected.

Total quantity passed in seven days $= \cdot 14112$ grm. or $\frac{1}{2 \cdot 2}$ of the total quantity injected.

2nd observation.—Injection of 5 c.c. of a 2 per cent solution of urea antimonyl tartrate ($= \cdot 03175$ grm. Sb).

| Day. | Amount of urine passed | Amount of antimony passed. |
|---|---|---|
| 1 | 525 c.c. | ·00330 grm. |
| 2 | 620 ,, | ·00220 ,, |
| 3 | 664 ,, | ·00159 ,, |
| 4 | 880 ,, | ·00153 ,, |
| 5 | 940 ,, | ·00140 ,, |
| 6 | 725 ,, | ·00126 ,, |
| 7 | ... | ·00108 ,, |

Total quantity of antimony passed in 24 hours $= \frac{1}{9 \cdot 6}$ of the quantity injected.

Total quantity of antimony passed in seven days $= \cdot 01236$ grm. or $\frac{1}{2 \cdot 5}$ of the total quantity injected.

3rd observation.—Injection of 5 c.c. of a 2 per cent solution of urea antimonyl tartrate ($= \cdot 03175$ grm. Sb).

| Day. | Amount of urine passed. | Amount of antimony passed. |
|---|---|---|
| 1 | 540 c c. | ·00468 grm. |
| 2 | 285 ,, | ·0021 ,, |
| 3 | 570 ,, | ·0024 ,, |
| 4 | 1,560 ,, | ·00132 ,, |
| 5 | ... | ·0012 ,, |
| 6 | 1,560 ,, | ·00108 ,, |
| 7 | 1,720 ,, | ·00081 ,, |

Total quantity of antimony passed in 24 hours $= \frac{1}{6{\cdot}8}$ of the quantity injected.

Total quantity of antimony passed in seven days $= {\cdot}01359$ grm. or $\frac{1}{2{\cdot}3}$ of the total quantity injected.

## Conclusions

(1) The proportion of the amount of antimony passed in 24 hours to the amount injected varies with the different antimonyl tartrates and in the following diminishing order :

(*i*) Lithium salt—urea salt.
(*ii*) Sodium salt—ammonium salt.
(*iii*) Potassium salt.

(2) The proportion of the amount of antimony passed in seven days after injection of an antimonyl tartrate to the amount injected tends to follow the above order approximately.

(3) The lithium salt is most quickly eliminated, next the urea salt, next the ammonium salt and lastly the potassium and sodium salts.

(4) Comparing the rate of solubility of the antimonyl tartrates, which is in the following order, *viz.*, (1) lithium salt, (2) sodium salt, (3) ammonium salt, (4) urea salt, (5) potassium salt, one finds that the amount excreted and the time taken by the urine to be free from antimony does not exactly follow the order of solubility of the salt. In the case of the urea and lithium salts, one has to consider their diuretic action.

(5) The curves of excretion of the different antimonyl tartrates have been described.

## (B)

## Excretion of Antimony after Repeated Injections of Tartar Emetic

It will be seen from our observations that only a small amount of antimony is excreted by the kidneys after intravenous injection of an antimonyl tartrate during the first twenty-four hours and that even after seven days there is still a fair proportion retained in the system. This fact opens up the question as to whether this retention of antimony by the system continues in the same proportion after repeated doses or whether after the antimony has reached a certain concentration in the system, it is excreted in larger quantities. To determine this we have investigated the excretion of antimony after repeated injections of tartar emetic and the following results were obtained :—

[*N.B.*—Since most of the antimony injected intravenously is excreted by the kidneys, only a little being passed by the stools, it may be assumed that the amount of antimony present in the tissues on a particular day is represented by the amount of antimony injected *minus* the amount of antimony excreted by the kidneys.]

### *1st Case*

1st injection—2½ c.c. of a 2 per cent solution of tartar emetic.
2nd injection—3 c.c. of a 2 per cent solution. 7th day.
} 2 injections.

| | | | |
|---|---|---|---|
| Amount of antimony excreted on | 1st day ... | ... ·00108 | grm. |
| Do. | 2nd ,, ... | ... ·00108 | ,, |
| Do. | 3rd ,, ... | ... ·00099 | ,, |
| Do. | 4th ,, ... | ... ·00054 | ,, |
| Do. | 5th ,, ... | ... ·000324 | ,, |
| Do. | 6th ,, ... | ... ·000324 | ,, |
| Do. | 7th ,, ... | ... ·002304 | ,, |
| Do. | 8th ,, ... | ... ·00162 | ,, |
| Do. | 9th ,, ... | ... ·00153 | ,, |
| Do. | 10th ,, ... | ... ·00144 | ,, |
| Do. | 11th ,, ... | ... ·00108 | ,, |
| Do. | 12th ,, ... | ... ·00099 | ,, |
| Do. | 13th ,, ... | ... ·000936 | ,, |

(1) Proportion of antimony excreted to the amount of antimony injected $= \frac{00108}{\cdot 018}$ or $\frac{1}{16 \cdot 7}$ on the 1st day.

(2) Total quantity of antimony excreted up to the 6th day = ·004338 grm.

Proportion of antimony excreted on the 7th day to the amount of antimony injected on the 7th day *plus* the amount present in the body due to the previous injection

$$= \frac{\cdot 002304}{\cdot 013662 + \cdot 0216} = \frac{1}{15 \cdot 3}.$$

## *2nd Case*

1st injection—2·5 c.c. of a 2 per cent solution of tartar emetic.
2nd injection—3 c.c. of a 2 per cent solution of tartar emetic on the 5th day from the beginning of observation.
3rd injection—4 c.c. of a 2 per cent solution of tartar emetic on the 8th day from the beginning of observation.
} 3 injections.

| | | |
|---|---|---|
| Amount of antimony excreted on the | 1st day ... ... | ·001296 grm. |
| Do. | 2nd ,, ... ... | ·00108 ,, |
| Do. | 3rd ,, ... ... | ·0009 ,, |
| Do. | 4th ,, ... ... | ·00063 ,, |
| Do. | 5th ,, ... ... | ·00282 ,, |
| Do. | 6th ,, ... ... | ·0028 ,, |
| Do. | 7th ,, ... ... | ·00162 ,, |
| Do | 8th ,, ... ... | ·0038 ,, |

(1) Proportion of antimony excreted to the amount of antimony injected on the first day $= \frac{\cdot 001296}{\cdot 018}$ or $\frac{1}{13 \cdot 9}$ of the amount injected.

(2) Total quantity of antimony excreted up to the 4th day = ·003906 grm.

Proportion of antimony excreted on the 5th day to the amount of antimony injected on the 5th day and the amount of antimony present due to the first injection $= \frac{\cdot 00282}{\cdot 014094 + \cdot 0216} = \frac{1}{12 \cdot 66}$ which is nearly equal to the proportion on the first occasion.

(3) Total quantity of antimony excreted up to the 7th day $= \cdot 011146$ grm.

Proportion of antimony excreted on the 8th day to the amount injected on the 8th day and the amount present due to the previous injections $= \frac{\cdot 0038}{\cdot 028454 + \cdot 0288} = \frac{1}{15 \cdot 3}$.

It will be seen from the above two observations that the amount of antimony passed by the kidneys was fairly proportional to the amount of antimony present in the tissues. In other words the law formulated by us (*Indian Journal of Medical Research*, January, 1924) that *the amount of antimony excreted by the kidneys was fairly proportional to the amount of antimony present* held good in those cases in which more than one injection was given.

Further observations proved that this law holds good only under certain limits and after several repeated injections of an antimonyl tartrate, a concentration is reached in the tissues at which the amount excreted by the kidneys is greater than what would follow from the above law, so that the concentration of the antimony in the tissues never exceeds this limit. We shall term this limit *maximum concentration limit* in the tissues. The tendency of the kidneys to throw out antimony from the tissues when it reaches a certain limit in the tissues is no doubt due to the mobility of the threshold of the kidneys, which is lowered when the above concentration is reached. This is illustrated in the following two observations :—

## *3rd Case*

1st injection—$2\frac{1}{2}$ c.c. of 2 per cent solution of tartar emetic.
2nd injection—3 ,, do. on the 4th day.
3rd injection—4 ,, do. do. 7th ,,
4th injection—5 ,, do. do. 11th ,,
5th injection—5 ,, do. do. 14th ,,
6th injection—5 ,, do. do. 17th ,,
7th injection—5 ,, do. do. 21st ,,

| | | |
|---|---|---|
| Amount of antimony excreted on the | 1st day ... ... | ·0009 grm. |
| Do. | 2nd ,, ... ... | ·00063 ,, |
| Do. | 3rd ,, ... ... | ·00065 ,, |
| Do. | 4th ,, ... .. | ·0015 ,, |
| Do. | 5th ,, ... ... | ·00103 ,, |
| Do. | 6th ,, ... ... | ·0018 ,, |
| Do. | 7th ,, ... ... | ·00594 ,, |
| Do. | 8th ,, .. ... | ·00504 ,, |
| Do. | 9th ,, ... ... | ·0029 ,, |
| Do. | 10th ,, ... ... | ·0031 ,, |
| Do. | 11th ,, ... ... | ·00684 ,, |
| Do. | 12th ,, ... ... | ·0036 ,, |
| Do. | 13th ,, .. ... | ·0036 ,, |
| Do. | 14th ,, ... .. | ·00738 ,, |
| Do. | 15th ,, ... ... | ·00828 ,, |
| Do. | 16th ,, ... ... | ·00612 ,, |
| Do. | 17th ,, ... ... | ·0117 ,, |
| Do. | 18th ,, ... ... | ·0087 , |
| Do. | 19th ,, ... ... | ·00576 ,, |
| Do. | 20th ,, ... ... | ·00648 ,, |
| Do. | 21st ,, ... ... | ·0134 ,, |
| Do. | 22nd ,, ... ... | ·0081 ,, |

(1) Proportion of antimony excreted on the 1st day to the amount injected $= \frac{\cdot 0009}{\cdot 018} = \frac{1}{20}$ of the amount injected.

(2) The total quantity of antimony excreted up to 3rd day is ·00218 grm. Sb.

The proportion of antimony excreted on the 4th day to the amount injected on the 4th day *plus* the amount of antimony already present in the body $= \dfrac{\cdot0015}{(\cdot018 - \cdot00218) + \cdot0216}$ $= \dfrac{1}{24\cdot9}$.

(3) The total quantity of antimony excreted up to 6th day is ·00651 grm. Sb.

The proportion of antimony excreted on the 7th day to antimony injected on the 7th day *plus* the amount of antimony already present in the body $= \dfrac{\cdot00594}{(\cdot0396 - \cdot00651) + \cdot0288}$ $= \dfrac{1}{10\cdot4}$.

(4) The total quantity of antimony excreted up to 10th day is ·02349 grm. Sb.

The proportion of antimony excreted on the 11th day to the antimony injected on the 11th day *plus* the amount present in the body is $= \dfrac{\cdot00684}{(\cdot0684 - \cdot02349) + \cdot036} = \dfrac{1}{11\cdot8}$.

(5) The total quantity of antimony excreted up to 13th day is ·03753 grm. Sb.

The proportion of antimony excreted on the 14th day to the antimony injected on the 13th day *plus* the amount present in the body is $= \dfrac{\cdot00738}{(\cdot1044 - \cdot03753) + \cdot036} = \dfrac{1}{13\cdot9}$.

(6) The total quantity of antimony excreted up to 16th day is ·05931 grm. Sb.

The proportion of antimony excreted on the 17th day to the antimony injected on the 17th day *plus* the amount present in the body is $= \dfrac{\cdot0117}{(\cdot1404 - \cdot05931) + \cdot036} = \dfrac{1}{10}$

(7) The total quantity of antimony excreted up to 20th day is ·09195 grm. Sb.

The proportion of antimony excreted on the 21st day to the amount injected on the 21st day *plus* the amount present in the body is $= \dfrac{\cdot 0134}{(\cdot 1764 - \cdot 09195) + \cdot 036} = \dfrac{1}{9}$.

### *4th Case*

| | | | |
|---|---|---|---|
| 1st injection of 2·5 c.c. | of 2 per cent solution | of tartar emetic. | |
| 2nd injection of 3 ,, | do. | on the 4th day. | |
| 3rd injection of 4 ,, | do. | do. 7th ,, | |
| 4th injection of 5 ,, | do. | do. 11th ,, | |
| 5th injection of 5 ,, | do. | do. 14th ,, | |
| 6th injection of 5 ,, | do. | do. 17th ,, | |
| 7th injection of 5 ,, | do. | do. 21st ,, | |

| | | | |
|---|---|---|---|
| Amount of antimony excreted on the | 1st day ... | ·001 | grm. |
| Do. | 2nd ,, ... | ·0009 | ,, |
| Do. | 3rd ,, ... | ·0005 | ,, |
| Do. | 4th ,, ... | ·00198 | ,, |
| Do. | 5th ,, ... | ·00171 | ,, |
| Do. | 6th ,, ... | ·00144 | ,, |
| Do. | 7th ,, ... | ·00288 | ,, |
| Do. | 8th ,, ... | ·00216 | ,, |
| Do. | 9th ,, ... | ·0023 | ,, |
| Do. | 10th ,, ... | ·0018 | ,, |
| Do. | 11th ,, ... | ·0046 | ,, |
| Do. | 12th ,, ... | ·0029 | ,, |
| Do. | 13th ,, ... | ·0019 | ,, |
| Do. | 14th ,, ... | ·0046 | ,, |
| Do. | 15th ,, ... | ·0063 | ,, |
| Do. | 16th ,, ... | ·00612 | ,, |
| Do. | 17th ,, ... | ·0123 | ,, |
| Do. | 18th ,, ... | ·0059 | ,, |
| Do | 19th ;, ... | ·0045 | ,, |
| Do. | 20th ,, ... | ·00648 | ,, |
| Do. | 21st ,, ... | ·01123 | ,, |
| Do, | 22nd ,, ... | ·0066 | ,, |

(1) The proportion of antimony excreted on the 1st day to the amount injected is $\frac{1}{18}$.

(2) The total quantity of antimony excreted up to 3rd day is ·0024 grm. Sb.

The proportion of antimony excreted on the 4th day to the amount injected on the 4th day *plus* the amount present in the body $= \frac{\cdot 00198}{(\cdot 018 - \cdot 0024) + \cdot 0216} = \frac{1}{18 \cdot 7}$.

(3) The total quantity of antimony excreted up to 6th day is ·00753 grm. Sb.

The proportion of antimony excreted on the 7th day to the amount injected on the 7th day *plus* the amount already present in the body $= \frac{\cdot 00288}{(\cdot 0396 - \cdot 00753) + \cdot 0288} = \frac{1}{21 \cdot 1}$.

(4) The total quantity of antimony excreted up to 10th day is ·01697 grm. Sb.

The proportion of antimony excreted on the 11th day to the amount injected on the 11th day *plus* the amount already present in the body $= \frac{\cdot 0046}{(\cdot 0684 - \cdot 01697) + \cdot 036} = \frac{1}{19}$.

(5) The total quantity of antimony excreted up to 13th day is ·02637 grm. Sb.

The proportion of antimony excreted on the 14th day to the amount injected on the 14th day *plus* the amount already present in the body $= \frac{\cdot 0046}{(\cdot 1044 - \cdot 02637) + \cdot 036} = \frac{1}{24 \cdot 7}$.

(6) The total quantity of antimony excreted up to the 16th day is ·04339 grm. Sb.

The proportion of antimony excreted on the 17th day to the amount injected on the 17th day *plus* the amount already present in the body $= \frac{\cdot 0123}{(\cdot 1404 - \cdot 04539) + \cdot 036} = \frac{1}{10 \cdot 8}$.

(7) The total quantity of antimony excreted up to the 20th day is ·07207 grm. Sb.

The proportion of antimony excreted on the 21st day to the amount injected on the 21st day *plus* the amount already present in the body $= \frac{\cdot 01123}{(\cdot 1764 - \cdot 07207) + \cdot 036} = \frac{1}{11 \cdot 4}$.

In the first case (third case in the paper) it will be seen that when the amount of antimony in the tissues reached (·1404 − ·05931) + ·036 grm., *i.e.*, ·11709 grm., the amount of its excretion is suddenly increased. In the second case (fourth case in the paper) when the amount of antimony in the tissues reached (·1404 − ·04539) + ·036, or ·13101 grm., then the same phenomenon was observed. In other words, it may be concluded that when the quantity of antimony present in the tissues exceeded these limits, the tendency towards concentration of the drug was prevented by a larger portion of antimony being excreted than that which could be excreted according to the law of excretion already referred to. These figures therefore represent the maximum concentration limit of antimony in the tissues of these individuals.

In the paper on the *Chemotherapy of Antimonial Compounds in Kala-azar Infection, Part VI* (*Indian Journal of Medical Research*, October, 1923), it was concluded that the results therein pointed to a cumulative action of tartar emetic or at least made the animal susceptible to the next higher dose. It appears from observations in the present paper that no cumulation takes place in the case of the drug above a certain limit, if it is injected at an interval of three or four days.

## Concentration of Antimony in Different Organs

A few observations have been made by us to determine the concentration of antimony in the different organs after

intravenous injection of tartar emetic in lethal and sub-lethal doses. So far it appears that the concentration of antimony is greatest in the liver. The following organs have been examined : (1) brain, (2) heart, (3) liver, (4) kidney, (5) the stomach and intestines.

## CONCLUSION

There is a *maximum concentration limit* of antimony in the tissues after repeated injections of tartar emetic, and this is the safeguard against any cumulation of the drug in the tissues when the concentration reaches this point. There is, however, a tendency towards accumulation of the drug in the tissues, after the first two or three injections.

We are much indebted to Dr. Bibhuty Bhushan Maity and Dr. Siris Chandra Banerjee for co-operating with us in our researches.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

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## PART XI

### THE VALUE OF UREA STIBAMINE IN THE TREATMENT OF EARLY KALA-AZAR

The therapeutic value of urea stibamine in the treatment of kala-azar has been established by the observations of Brahmachari, Shortt, Shortt and Sen, Foster and others. This antimonial preparation has recently been given an extensive trial in the wards of the Medical College Hospital, Calcutta.

To Lieut.-Colonel Barnardo, I.M.S., Principal and Professor of Medicine, and Lieut.-Colonel McCay, I.M.S., Professor of Clinical Medicine at the Calcutta Medical College, as well as to Major Shortt, I.M.S., Special Kala-azar Research Officer, Shillong, and Dr. Percy Foster, Medical Officer, Badlipar Tea Estate, I am under deep obligation for their kind courtesy in supplying me with the records of their observations in a series of cases and allowing me to make use of them in this communication.

In my paper on the *Chemotherapy of Antimonial Compounds in Kala-azar Infection, Part IX*, I have given my observations in the treatment of *resistant* cases of kala-azar with urea stibamine. The present paper is intended to give an account of its value in *early* cases of the disease. From an economic point of view, it seems desirable that its value should be assessed in such early cases to determine whether

sterilization can be brought about in them more quickly than in chronic cases.

By *early* cases I mean cases in their first attack of fever or in which the duration of the disease did not generally extend more than four months.

The following series of cases show the remarkable rapidity with which sterilization was obtained in them. To give an idea of the observations made by different observers, I have given here some of the cases under the observers mentioned above, in addition to some of mine.

## I. Medical College Series

(1) Patient P., European male, æt. 35 years, was admitted into the wards of Lieut.-Colonel Barnardo, I.M.S., with history of fever of about a week's duration. He had a previous attack of typhoid-like fever two months before, lasting for about three weeks. Temperature ranged from 102°F. to 103°F. with double rises and the spleen extended 2″ below the costal arch. There was a progressive leucopenia, the leucocytes diminishing from 3,750 to 2,200 in seven days. Blood culture and Widal reaction for *B. typhosus* were negative and no malarial parasites were found.

The diagnosis of kala-azar having been made, patient was given intravenous injection of urea stibamine twice a week, in doses of 0·1 to 0·2 gramme in five injections in the course of a fortnight. Total quantity—0·55 gramme. After three injections temperature came down to normal and the spleen could hardly be felt below the costal arch. Blood culture on N.N.N. medium was negative after the 5th injection. Leucocyte count: (1) Before treatment—2,200 ; (2) after treatment—6,000.

(2) Patient M., æt. 10, European female, was admitted into the wards of Lieut.-Colonel Barnardo, for treatment of remittent fever of three months' duration. Temperature

ranged from 102° to 104°F. with double rise, the spleen extending up to the umbilicus. There was a progressive leucopenia, the leucocytes diminishing from 2,600 to 2,200 in seven days' time. Blood culture for *B. typhosus* and Widal reaction were negative and no malarial parasites were found.

The diagnosis of kala-azar having been made, patient was given intravenous injection of urea stibamine in doses of 0·05 to 0·15 gramme in seven injections in course of two weeks. Total quantity—0·8 gramme. The temperature came down to normal after the 4th injection. The blood culture on N.N.N. medium for flagellates was negative after the 6th injection. At the time of discharge, which was three weeks after treatment was stopped, the spleen and liver could not be felt below the costal arch and blood culture for flagellates was negative. Leucocyte count: (1) Before treatment—2,200 ; (2) after treatment – 6,000.

(3) Patient M., European male, æt. 7, was admitted into the wards of Lieut-Colonel Barnardo, I.M.S., for treatment of remittent fever of 18 days' duration. Temperature ranged from 100°F. to 103°F., the spleen extending 2″ below the costal arch. Widal reaction and blood culture for *B. typhosus* were negative. Leucocyte count—2,500. Peripheral blood culture on N.N.N. medium for flagellates was positive.

The diagnosis of kala-azar having been made, the patient was given intravenous injection of urea stibamine in doses of 0·05 to 0·1 gramme in 5 injections in two weeks. Total quantity—0·35 gramme. Temperature came down to normal after two injections, the leucocyte count, after the treatment was stopped, was 6,250. Blood culture for flagellates was negative after 5 injections. Spleen disappeared below the costal arch after 5 injections. At the time of discharge, blood culture for flagellates was negative. Leucocyte

count: (1) Before treatment—2,200; (2) after treatment—8,750.

(4) Patient Miss S., æt. 33, was admitted into the wards of Lieut.-Colonel McCay, I.M.S., with history of fever of about 10 days' duration. On admission spleen was just palpable below the costal arch. Peripheral blood culture on N.N.N. medium showed the presence of flagellates.

The patient was given intravenous injection of urea stibamine, the doses ranging from 0·05 to 0·15 gramme in 6 injections during 10 days. Total quantity 0·7 gramme. Temperature came down to normal after the 3rd injection and the peripheral blood culture for flagellates was negative after the 5th injection. One month after the treatment was stopped the spleen was not palpable below the costal margin. Leucocyte count: (1) Before treatment—3,200; (2) after treatment—6,000.

(5) Patient Miss S., æt. 15, was admitted into the wards of Lieut.-Colonel McCay, I.M.S, with history of fever of four days' duration. On admission spleen was just palpable below the costal margin, the leucocyte count being 3,800. Peripheral blood culture for flagellates on N.N.N. medium was positive.

The patient was given intravenous injection of urea stibamine, the dose ranging from 0·05 to 0·1 gramme in 4 injections during ten days. Total quantity—0·3 gramme. Temperature came down to normal and remained so after 3 injections. The blood culture for flagellates on N.N.N. medium was negative after 4 injections. Leucocyte count: (1) Before treatment—3,800; (2) after treatment—6,500.

(6) Patient E. M., æt. 15, was admitted into the wards of Lieut.-Colonel McCay, I.M.S., with history of fever of three weeks' duration. Patient was in hospital for about a month during which she was found to suffer from remittent fever with double rise and spleen extended ½″ below the umbilicus. Peripheral blood was cultured for flagellates

the result being positive. Patient was treated with intravenous injection of urea stibamine twice a week, the dose ranging from 0·05 to 0·2 gramme in 8 injections. Total quantity—0·9 gramme. Fever stopped after the 3rd injection and spleen disappeared completely below the costal arch after the 7th injection. Blood culture was found negative after the 8th injection. Leucocyte count: (1) Before treatment—3,600; (2) after treatment—6,700.

(7) Patient æt. 8, H. M., came under my treatment with history of fever for about two months and an attack of remittent type of fever four months previously. The spleen was three fingers below the costal arch. Peripheral blood culture on N.N.N. medium showed the presence of flagellates and the blood picture was—R.B.C.—3,000,000, W.B.C.—3,500, Hb.—60 per cent.

Patient was treated with intravenous injection of urea stibamine, the doses ranging from 0·05 gramme to 0·1 gramme, the number of injections being six in the course of 12 days. Total quantity—0·4 gramme. After two injections the temperature came down to normal and the spleen could hardly be felt below the costal arch after the 5th injection. Blood culture was negative after the 6th injection. Blood picture: (1) Before treatment—R.B.C.—3,000,000, W.B.C.—3,500, Hb.—60 per cent; (2) after treatment—R.B.C.—4,500,000, W.B.C.—7,500, Hb.—75 per cent.

(8) Patient named D., æt. 25, came under my treatment with history of high remittent fever of eight days' duration, temperature ranging between 103° and 104°F. The spleen could just be felt below the costal arch. Widal reaction for typhoid was positive 1 in 20. Peripheral blood culture on N.N.N. medium showed flagellates. Patient was treated with intravenous injection of urea stibamine in doses of 0·1 gramme each. Altogether 5 injections were given in ten days. Total quantity—0·5 gramme. The fever stopped after the 1st injection. Blood culture on N.N.N. medium

was negative after the 4th injection. Blood picture: (1) Before treatment—R.B.C. 3,200,000, W.B.C.—3,600, Hb. 46 per cent.; (2) after treatment—R.B.C. 4,600,000, W.B.C.—9,000, Hb.—70 per cent. Two months after completion of treatment, blood culture for flagellates was negative.

(9) Patient named C., æt. 25, H. M., came under my treatment with history of fever for 10 days. The fever was of high remittent type and the patient was drowsy for four days. Spleen could just be felt below the costal arch. Peripheral blood culture on N.N.N. medium showed flagellates. Patient was given intravenous injections of urea stibamine in doses ranging between 0·05 to 0·1 gramme in the course of 14 days. Altogether 0·5 gramme was given in 6 injections. The fever stopped after the 2nd injection and blood culture became negative after the fourth. Blood picture: (1) Before treatment—R.B.C.—3,600,000, W.B.C.—1,400, Hb.—42 per cent; (2) after treatment—R.B.C.—4,300,000, W.B.C.—6,400, Hb.—50 per cent. Blood culture was negative two months after completion of treatment.

(10) Patient named R., æt. 20, H. M., came under my treatment with history of high remittent fever for 21 days. Spleen could just be felt below the costal arch. Peripheral blood culture on N.N.N. medium showed flagellates. Patient was given intravenous injection of urea stibamine, the dose ranging from 0·05 to 0·15 gramme. Altogether 6 injections were given in the course of 14 days. Total quantity—0·55 gramme. The fever stopped after the 3rd injection and the blood culture became negative after the 5th injection. Blood picture: (1) Before treatment—R.B.C.—3,600,000, W B.C.—2,400, Hb.—36 per cent; (2) after treatment—R.B.C.—4,500,000, W.B.C.—8,200, Hb.—60 per cent. Blood culture was negative two months and a half after completion of treatment.

(11) Patient, named D., came under my treatment with history of fever of three weeks' duration. Spleen extended three fingers below the costal arch. Blood culture for flagellates was positive. The patient was given intravenous injection of 0·1 gramme of urea stibamine on 22-5-1924 and on 27-5-1924. Fever stopped after the first injection and spleen could not be felt below the costal arch after the second injection. Blood picture: (1) Before treatment—R.B C.—2,300,000, W.B.C.—3,100, Hb.—60 per cent; (2) after treatment—R.B.C.—5,000,000, W.B.C.—6,000, Hb.—85 per cent. Blood culture—negative on 28-6-1924.

## II. Cases in Pasteur Institute, Shillong (Major Shortt, I.M.S.)

| Sex / Age. | Duration of illness at commencement of treatment. | Before and After Completion of Treatment with Urea Stibamine. | | | | No. of injections and total amount of urea stibamine. | Duration of treatment in days. | Increase in weight in lbs. after completion of treatment. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| | | Size of spleen. | Spleen puncture. | Blood picture. | Blood culture. | | | | |
| M. / 21 yrs. | $4\frac{1}{2}$ months | Up to navel / Not felt below ribs | Positive / Negative | Hb. $\frac{60}{80}$ R.B.C. $\frac{4,350,000}{6\,030,000}$ W.B.C. $\frac{2,600}{9,000}$ | Positive / Negative | 0·7 gramme in 4 injections. | 7 | $13\frac{1}{2}$ lbs. | Patient cured. Patient was previously treated with 43 c.c. of one per cent solution of sodium antimonyl tartrate without benefit. |
| M. / 15 yrs. | 2 months | 3 fingers' breadth / Just palpable | Positive / Negative | Hb. $\frac{60}{75}$ R.B.C. $\frac{3,512,000}{5,660,000}$ W.B.C. $\frac{4,000}{15,000}$ | × / Negative | 1·7 grammes in 8 injections. | 20 | 15 lbs. | Patient cured. |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| M. / 16 yrs. | 3 months | 4 fingers to the left and 3 above umbilicus / Not felt below ribs | Positive / Negative | Hb. 65/85 R.B.C. 5,960,000/6,000,000 W.B.C. 9,000/10,400 | Positive / Negative | 0·75 gramme in 5 injections. | 7 | 13 lbs. | Patient cured. Patient was previously treated with 55 c.c. of one per cent solution of sodium antimonyl tartrate without benefit. |
| M. / 28 yrs. | 5 months | Below umbilicus / Up to umbilicus | Positive / Negative | Hb. 60/85 R.B.C. 3,400,000/5,100,000 W.B.C. 4,000/9,600 | × / Negative | 0·7 gramme in 8 injections. | 15 | 17 lbs. | Patient cured. |

## III. Badlipar Series of Cases (Dr. Percy Foster)

| Sex / Age. | Duration of illness at commencement of treatment. | Before and After Completion of Treatment with Urea Stibamine. Size of spleen. | Spleen puncture. | No. of injections and total amount of urea stibamine. | Remarks. |
|---|---|---|---|---|---|
| M. / 5 yrs. | 2 months | 3½ fingers below costal arch / 3 fingers | Positive / Negative | 0·41 gramme in 5 injections. | Patient cured. Patient was previously treated with 1·5 grammes of sodium antimonyl tartrate without any benefit. |
| M. / 21 yrs. | ½ month. | 3½ fingers below costal arch / Nil | Positive / × | 0·9 gramme in 5 injections. | Patient cured. |
| F. / 6 yrs. | 1 week | 1½ fingers below costal arch / Nil | Positive / × | 0·35 gramme in 4 injections | Patient cured. |
| M. / 28 yrs. | 3 months | 5 fingers below costal arch / Nil | Positive / × | 2·15 grammes in 10 injections. | Patient cured. |
| M. / 35 yrs. | 15 days. | 5 fingers below costal arch / 3 fingers | Positive / Negative | 2·05 grammes in 10 injections. | Patient cured |
| M. / 49 yrs. | 1 month | 2 fingers below costal arch / Nil | Positive / Negative on liver puncture | 1·70 grammes in 8 injections | Patient cured. |

## Remarks

It is evident from the cases recorded above that urea stibamine cuts short the course of kala-azar to a remarkable degree, if administered in its early stages. The same conclusions have been arrived at from observations made by different observers in different places. That the drug cuts short the course of the disease in all its stages has already been shown by previous observations. Its beneficial effects in its early stages are however most noteworthy.

In the early cases blood culture was most helpful in the diagnosis of the disease.

It is evident from an economic point of view and in the interests of the miserable sufferers, that the use of urea stibamine in the early stages of the disease cannot be over-emphasised.

I am indebted to my assistants—Sub-Assistant Surgeon Bibhuty Bhusan Maity for making flagellate cultures and Sub-Assistant Surgeon Sirish Chandra Banerjee for the blood count, of my cases described in the present paper.

[Reprinted from the Indian Journal of Medical Research, Vol. XII, No. 2, October, 1924]

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, June 16, 1924]

## PART XII

### SOME OBSERVATIONS ON THE CONSTITUTION OF UREA STIBAMINE AND STIBAMINE

Urea Stibamine is the name given (Brahmachari [1]) to the compound formed by treating *p*-aminophenyl-stibinic acid with urea.

The constitution of this compound originally suggested was urea salt of *p*-amino-phenyl-stibinic acid or

$$CO\,(NH_2)_2\,.\,NH_2\,.\,C_6H_4\,.\,Sb \begin{cases} OH \\ =O. \\ OH \end{cases}$$

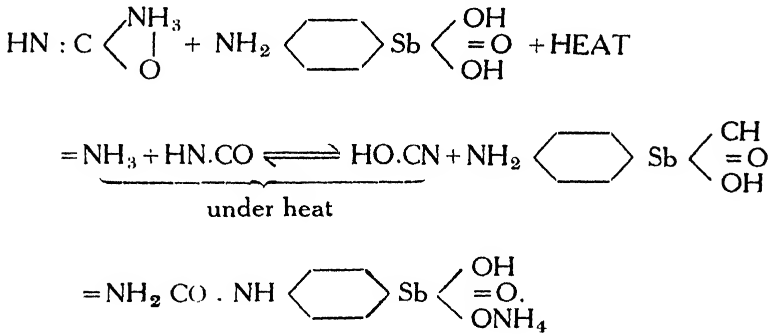
In our subsequent research, it was observed that when an aqueous solution of urea stibamine is treated with dilute hydrochloric acid, a precipitate is obtained which does not dissolve in excess of dilute hydrochloric acid. This precipitate is evidently different from *p*-amino-phenyl-stibinic acid which dissolves in excess of dilute hydrochloric acid. This fact led us to review the constitution of urea stibamine as originally suggested and this constitutes the basis of the present paper.

When urea in aqueous solution is heated above 60°C, cyanic acid is formed as is shown by appearance of a precipitate which is formed on addition of silver nitrate to the solution of urea and which is insoluble in water. The conversion of urea into cyanic acid is best explained by the

constitution of urea as suggested by Werner and the decomposition may be explained by a simple and straightforward change, thus :—

$$HN : C \left\langle \begin{matrix} NH_3 \\ - \\ O \end{matrix} \right. = \begin{matrix} NH_3 \\ + \\ HNCO \end{matrix} \rightleftharpoons HO \cdot CN.$$

When urea is heated with *p*-amino-phenyl-stibinic acid, instead of the urea forming a salt with the acid, the cyanic acid formed may combine with its $NH_2$ radicle giving rise to a carbamino derivative of the acid, the $NH_3$ formed at the same time combining with its acidic portion. The reaction may be expressed as follows :—




The decomposition of urea into cyanic acid and ammonia does not take place on heating a solution of urea in excess of methyl alcohol and therefore the formation of the above carbamino derivative should not take place when a solution of urea in methyl alcohol is treated with *p*-amino-phenyl-stibinic acid, even with the aid of heat. Experimentally this has been found by us to be the case. Thus when a solution of urea in methyl alcohol is treated with *p*-amino-phenyl-stibinic acid, the latter goes into solution probably giving rise to the formation of a compound having the structure of urea stibamine as originally suggested by one of us. This compound, if formed at all, must be a very feeble one, because when the above solution is treated with

an excess of absolute alcohol, the original *p*-amino-phenyl-stibinic acid is precipitated and urea goes into solution.

Further experimental evidence in favour of the structure of urea stibamine suggested by us here is given by various chemical properties of urea stibamine and by its quantitative analysis.

*Purification of urea stibamine.*—

2 grammes of urea stibamine prepared in the usual way are dissolved in the least quantity of water and the solution filtered. To the filtrate absolute alcohol is added and the precipitate formed is filtered and washed with absolute alcohol till free from urea. It is then dried *in vacuo* over fused calcium chloride.

*Composition.*—

Calculated from $NH_2.CO.NH.C_6H_4.SbO.OH.ONH_4.H_2O$.

C=24·7, H=3·23, N=12·3, Sb=35·3 per cent.

Found C=24·6, H=4·37, N=12·52, Sb=35·09 per cent.

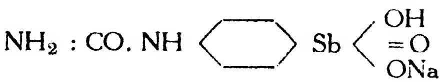
*Chemical properties of urea stibamine.*—

(1) To an aqueous solution of urea stibamine add dilute hydrochloric acid ; a precipitate forms insoluble in excess of the acid (distinction from *p*-amino-phenyl-stibinic acid).

(2) To an aqueous solution of the substance add alcoholic caustic potash and chloroform ; on heating no smell of phenyl-isocyanate is obtained.

(3) One gramme of urea stibamine is dissolved in water and to this strong cooled hydrochloric acid is gradually added till a precipitate that is at first formed is dissolved. After cooling the solution 0·2 gramme of sodium nitrite dissolved in water is added to it. The mixture is now treated with alkaline $\beta$-naphthol solution. No dye formation takes place.

From the above tests, it is evident that urea stibamine contains no $NH_2$ group attached to the benzene nucleus.

Preparation of 

or sodium salt obtained by the replacement of $NH_4$ from urea stibamine.

## EXPERIMENTAL

2 grammes of urea stibamine are dissolved in water and to the cooled solution dilute hydrochloric acid is added in excess. The precipitate is washed several times with dilute hydrochloric acid and subsequently with distilled water till it is free from hydrochloric acid. The washed precipitate is now dissolved in sodium hydroxide solution and the solution subsequently neutralized with dilute acetic acid. The solution is filtered and to the filtrate is added an excess of absolute alcohol till complete precipitation takes place. The precipitate is washed several times with absolute alcohol and then dried *in vacuo* in a desiccator over fused calcium chloride.

*Composition.*—

Calculated from $NH_2.CONH.C_6H_4.SbO.OH.ONa$.

$C = 25{\cdot}54$, $H = 2{\cdot}4$, $N = 8{\cdot}51$ per cent.

Found $C = 25{\cdot}4$, $H = 2{\cdot}6$, $N = 8{\cdot}4$ per cent.

*Chemical properties.*—

(1) It is insoluble in dilute hydrochloric acid.

(2) It does not show the presence of free $NH_2$ (*see* properties of urea stibamine).

(3) It does not give rise to dye formation (*see* above).

Both urea stibamine and the above sodium salt on chemical analysis correspond to the formula that they are carbamino derivatives. In the case of urea compound, the

formula corresponds to the carbamino compound with one molecule of $H_2O$ added to it.

## STIBAMINE

This is the sodium salt of *p*-amino-phenyl-stibinic acid and the name was given to the compound by me (*Journal of Tropical Medicine and Hygiene*, August 15th, 1921).

It appears that in the formation of the sodium salt three molecules of *p*-amino-phenyl-stibinic acid polymerize with separation of two molecules of water. The sodium salt of the polymerized acid is stable and neutral in its reaction when dissolved in water.

The formula for the polymerized acid will be $(NH_2 C_6H_4 SbO)_3 H_2O_3$.

## EXPERIMENTAL

Stibamine is prepared by carefully dissolving *p*-amino-phenyl-stibinic acid in a solution of sodium hydroxide.

*Composition.*—

Calculated for $(NH_2 C_6H_4 SbO)_3 HO_3Na$.

C=28·3 per cent, H=2·3 per cent, Sb=47·9 per cent.

Found C=27·69 per cent, H=2·89 per cent, Sb=47·4 per cent.

## REMARKS

1. In the light of more recent investigations, the constitution of urea stibamine has to be modified from what was originally suggested.

2. Unlike stibamine it does not undergo polymerization.

3. Another allied aromatic antimonial compound that does not undergo polymerization is the sodium salt of amido-glycine-*p*-amino-phenyl-stibinic acid, which was

named (Brahmachari [2]) stibglycine-amide ($NH_2$. CO. $CH_2$. NH. $C_6H_4$. SbO. OH. ONa).

Further work on the reactions between urea and the ortho or para-amino-aromatic acids is in progress in our laboratory.

My grateful thanks are due to Major Boyd, I.M.S., Chemical Examiner to the Government of Bengal, for kindly giving my assistant Mr. Das facilities for conducting the combustion experiments in his laboratory in connection with the present research.

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1 BRAHMACHARI, U. N. : (1922) *Ind. Journ. Med. Res*, Vol. X, No. 2, p. 492.
2 BRAHMACHARI, U. N. : (1922) *Ind Journ. Med. Res.*, Vol. X, No 2, p. 492.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, August 4, 1924]

## PART XIII

### FURTHER OBSERVATIONS ON DERMAL LEISHMANOID

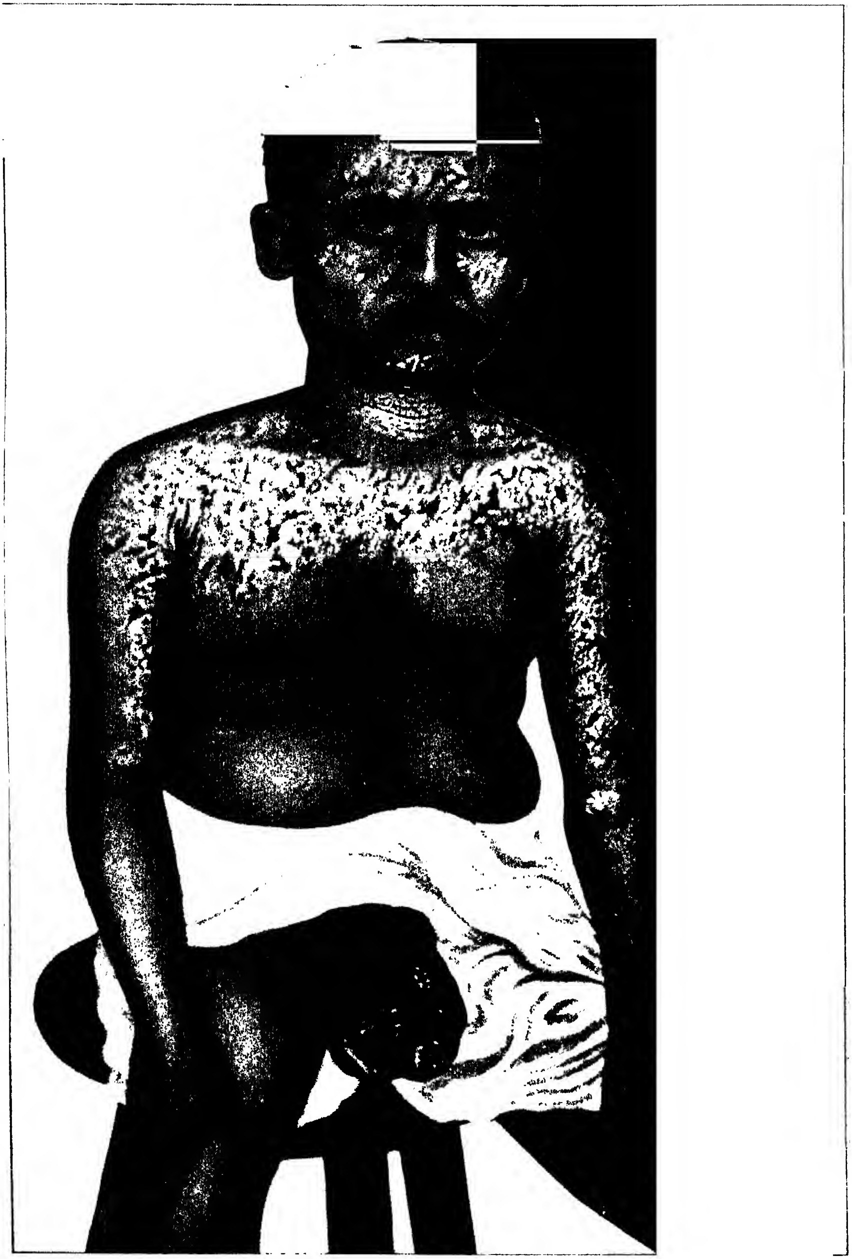
In the *Indian Medical Gazette* (1922) and in the *Indian Journal of Medical Research* (1923), an account was given of the remarkable skin infection which is observed very rarely in patients suffering from kala-azar after they have undergone a course of antimonial treatment and have been apparently cured as far as the infection of the internal organs is concerned.

Recently a similar condition was discovered in another patient under almost similar conditions.

*Previous history.*—About two years and a half ago, the patient, æt. 45, came under the treatment of Brahmachari. He presented all the symptoms of kala-azar, e.g., double rise of temperature, enlargement of the spleen and liver. Blood picture : R.B.C.—2,700,000, W.B.C.—2,400, Hb.—34 per cent. He was treated with 2 per cent solution of sodium antimonyl tartrate, 26 injections being given in doses of ½ to 5 c.c. twice a week. When he stopped treatment, his condition was as follows : R.B.C.—3,600,000, W.B.C.—4,500, Hb.—46 per cent, spleen 2″ below the costal arch, no rise of temperature for nearly a month.

[*Reprinted from the Indian Journal of Medical Research, Vol. XII, No. 3, January, 1925*]

PLATE XXXII



**Photograph of a patient showing peculiar eruption of the body nine months after completion of the second course of treatment—a case of Dermal Leishmanoid. (Vide para. 21.**

Six months later, he began again to suffer from fever with increase in the size of the spleen and bleeding from the gums. Blood picture : R.B.C.—2,600,000, W.B.C.—1,200, Hb.—36 per cent. He was evidently suffering from a relapse as shown by a positive flagellate culture from the peripheral blood. He was treated with another course of sodium antimonyl tartrate, 20 injections being given, when he was apparently cured.

Nine months after completion of the second course of treatment, he noticed a peculiar eruption on his body. The lesions appeared at first as two or three nodules over the ear. These subsequently increased in size and other nodules appeared over the skin in different parts of the body. Along with these a number of slightly raised brown patches appeared over the body, none of which were anæsthetic (*see* Plate XXXII). There was no enlargement of the spleen or the liver. Blood condition : R.B.C.—3,900,000, W.B.C.—7,800, Hb.—75 per cent. No flagellates could be developed on culture of peripheral blood from the veins. Scrapings from the nodule showed the presence of L. D. bodies and culture of the juice from the nodules gave positive findings.

The histo-pathological changes in the skin are described below by Shortt and the location of the parasites with regard to the layers of the skin is indicated.

The patient was put on a course of treatment with urea stibamine. Altogether 20 injections of the compound were given intravenously in doses of ·1 to ·2 grm. After the treatment, the nodules diminished much in size and the patches on the skin diminished to a great extent. After 15 injections had been given the scrapings from the nodules did not show the presence of definite L. D. bodies, but a few suspicious-looking bodies were found. The patient stopped treatment when he had improved to a great extent. He was accidentally discovered by Brahmachari three

months after the treatment was stopped, when he appeared to have still more improved but a few nodules and some patches were still present. No opportunity was given on this occasion to examine scrapings from the skin. The general conclusion is that the patient had benefited to a great extent. During the whole period the patient was under treatment for his skin lesions, no signs of internal leishmaniasis were ever observed. Apparently, the *leishmania* affecting the skin of the patient were not absolutely antimony-fast as shown by the improvement in the skin condition under treatment with urea stibamine. The rather slow improvement of the skin makes one think that the parasites were probably more resistant to antimony than ordinary *leishmania* or were possibly less accessible to the drug than when situated in other tissues. In other words, the case appears to be one of infection of the skin by *leishmania* rendered somewhat resistant to antimony by previous antimonial treatment of the case.

## HISTO-PATHOLOGY

BY

MAJOR H. E. SHORTT, I.M.S.

*Pasteur Institute, Shillong.*

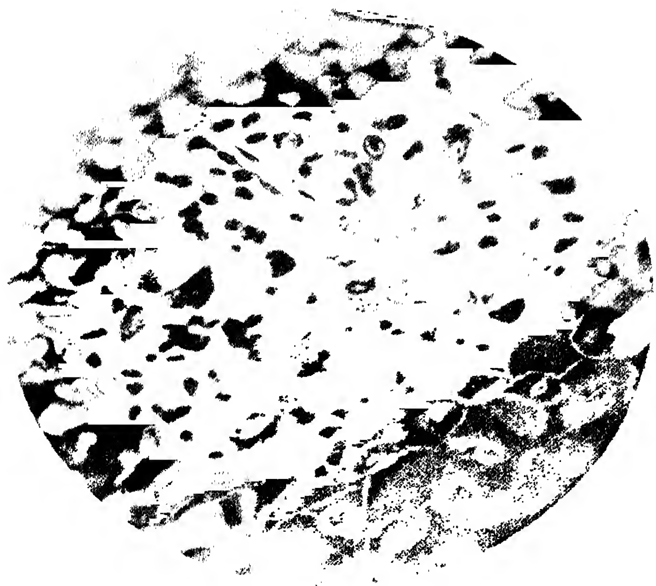
In 1922, Brahmachari described an interesting condition involving multiple skin lesions in a treated case of kala-azar. Since then this condition has been found to be of very rare occurrence, but I had the privilege of seeing a similar, though less marked, case which came under his treatment. I am indebted to him for the tissues from which the present description has been prepared.

The lesions, which took the form of papules varying from $\frac{1}{8}''$ to $\frac{1}{2}''$ in diameter, were most numerous in the scrotal region, but were also distributed over the trunk, limbs

Serial No. 20

[*Reprinted from the Indian Journal of Medical Research, Vol. XII, No. 3, January, 1925*]

PLATE XXXIII



FIG, 2

Section showing appearance of epidermis and cutis vera in superficial tissue from nodule of a Deimal Leishmanoid case under high power. (Vide para. 5).

and head. The description given below applies to a section of one of these nodules cut in a direction perpendicular to the surface of the skin.

### Appearances under a Low Power (Plate XXXIII, Fig. 1)

The first glance shows that there is a profound alteration in the structure of the superficial tissues. This alteration implicates both the epidermis and the *cutis vera* and these will be considered separately.

*Epidermis.*—The structural modification here consists in a uniform attenuation of the epidermis affecting all the recognised layers, but most evident in the *rete mucosum* on account of the relative diminution in number and length of the finger-like processes usually associated with this layer in normal skin. While the thickness of the epidermis is thus greatly reduced, there is, at the same time, no tendency for this process to proceed to the extreme degree of ulceration.

*Cutis vera.*—It is in this region that the most striking changes are manifest. The normal condition of dense connective tissue, merging gradually into the more open subcutaneous tissue, is entirely replaced by what appears to be a dense infiltration of cells, forming a deep layer sharply differentiated from the underlying open structure of the subcutaneous tissue. This cellular layer averages in depth about nine times the depth of the epidermis.

### Appearances under a High Power (Plate XXXIII, Fig. 2)

*Epidermis.*—Beyond a diminution in the thickness of the layers the minute structure is unaltered.

*Cutis vera.*—This appears to be composed of a very primitive connective tissue more or less uniform throughout its extent, and formed by, rather than infiltrated with, irregularly shaped cells, many of which have branching

processes. The nuclei of the cells are irregularly oval in shape, and stain deeply. The tissue is very vascular and new formation of blood capillaries is actively in progress. The cells composing the walls of the smallest capillaries do not always appear to be distinct from the stroma cells and the impression received from a careful examination of the tissue is that both capillary endothelium and stroma cells are derived from the same primitive undifferentiated cell.

This appears to be the cell which almost invariably contains the intracellular forms of *Herpetomonas donovani* which are the cause of the condition. Once the primitive cell has become definitely differentiated into a stroma cell or into capillary endothelium, it seems to lose its power of active phagocytosis. The parasitised cells are mainly situated close under the epidermal layer and become fewer in number in the deeper parts of the tissue. Many of these cells, besides containing parasites, enclose abundant pigment granules as the tissue was taken from an Indian patient. The connective tissue underlying the *cutis vera* is quite normal in appearance.

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[Reprinted from the Indian Journal of Medical Research, Vol. XII, No. 4, April, 1925]

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, November 17, 1924]

## PART XIV

### OBSERVATIONS ON A SERIES OF CASES OF KALA-AZAR TREATED WITH UREA STIBAMINE DURING A COURSE OF 32 HOURS TO 7 DAYS

In the present paper are described a series of cases of kala-azar either under our observation or that of others in which sterilization appears to have been brought about in seven days or less by treatment with urea stibamine. Our grateful thanks are due to Major Shortt, I.M.S., Lt.-Col. Greig, I.M.S., Dr. Kundu, Dr. Percy Foster and Dr. Banerjee, Badlipar, Assam, for supplying Brahmachari with notes of their cases. By sterilization is meant either negative spleen puncture or negative culture from splenic material or from the peripheral blood, together with subsequent disappearance of clinical symptoms of the disease.

### *Cases of Major Shortt, I.M.S. (Pasteur Institute, Shillong)*

(1) Patient, æt. 21, male. Fever for four months and a half. Spleen puncture—positive. Urea stibamine, 0·7 grm., given in four injections during seven days. Sterilization proved by negative microscopic and cultural examination of the splenic juice.

(2) Patient, æt. 16, male. Fever for three months. Spleen puncture—positive. Urea stibamine, 0·75 grm., given in five injections during seven days. Sterilization proved by negative microscopic and cultural examination of the splenic juice.

(3) Patient, æt. 16, male. Fever for eight months. Spleen puncture—positive. Urea stibamine, 0·65 grm., given in five injections during six days. Sterilization proved by negative microscopic and cultural examination of the splenic juice.

## *Cases of Lt.-Col. Greig, I.M.S. and Dr. Kundu (Pasteur Institute, Shillong)*

(4) Patient No. 73.—Admitted on 2nd June. Spleen puncture on 4th June. L. D. bodies + + +. Culture + + + on 10th June. Urea stibamine, first injection, 0·2 grm., 9th June. Second injection, 0·25 grm., 11th June. Third injection, 0·25 grm., 13th June. Total quantity—0·7 grm.

Spleen puncture on the 13th June, on the last day of injection. L. D. bodies—*nil*. Culture—negative after 10 days, that is, on 23rd June.

Actual period between the first and third injections—five days. Period of illness—four months.

(5) Patient No. 74.—Admitted on 2nd June, 1924. Spleen puncture on 4th June, 1924. L. D. bodies + +. Culture + + + 10th June. First injection, urea stibamine, 0·2 grm., 9th June. Second injection, 0·25 grm., 11th June. Third injection, 0·25 grm., 13th June. Total quantity—0·7 grm.

Spleen puncture on 13th June, on the last day of injection. L. D. bodies—*nil*. Culture—negative on 23rd June.

Actual period between the first and third injections—five days. Period of illness—two months.

(6) Patient No. 164.—Admitted on 21st September, 1924. Spleen puncture on 22nd September. L. D. bodies—*nil*. Culture—negative, 1st October. First injection, urea stibamine, 0·2 grm., 24th September. Second injection, 0·25 grm., 26th September. Third injection, 0·25 grm., 26th September. Fourth injection, 0·25 grm., 30th September. Total quantity—0·95 grm.

Spleen puncture on 1st October. L. D. bodies—*nil*.

Culture—negative after ten days, that is, on the 11th October. Actual period between first and fourth injections—seven days. Period of illness—one year and five months.

(7) Patient No. 171.—Admitted on 25th September. Liver puncture, 27th September. L. D. bodies—*nil*. Culture—positive on 8th October, 1924.

First injection of urea stibamine, 8th October—0·2 grm. Second injection, 10th October—0·25 grm. Third injection, 12th October—0·25 grm. Fourth injection, 14th October—0·25 grm. Total quantity, 0·95 grm.

Liver puncture on 16th October. L. D. bodies—*nil*. Culture—negative, 24th October, 1924.

Actual period between first and fourth injections—seven days. Period of illness—about three months.

*N.B.*—These four cases, the notes of which have been very kindly forwarded to Brahmachari, are referred to in a joint paper by Lt.-Col. Greig, I.M.S. and Dr. Kundu published in the present number.

## *Cases of Dr. Percy Foster and Dr. Banerjee (Badlipar, Assam)*

(8) Patient, æt. 6, female. Fever for seven days. Spleen puncture—positive. Urea stibamine, 0·35 grm., given in four injections. Sterilization proved by disappearance of the clinical symptoms and subsequent history of the patient.

(9) Patient, æt. 6, female. Fever for fifteen days. Spleen puncture—positive. Urea stibamine, 0·5 grm., given in four injections during seven days. Sterilization proved by negative microscopic examination of the splenic juice and subsequent history of the patient.

## *Cases of Brahmachari and Maity*

(10) Patient, æt. 14 months. History of illness—three months. Spleen 3″ and liver 2″ below costal arch. Temperature—100° to 102°F. Blood culture—positive. Urea stibamine, 0·05 and 0·1 grm., given intravenously in four days (two injections in all). Temperature came down to normal after first injection. Spleen could not be felt below costal arch after last injection. Actual period between first and last injections—three days. Peripheral blood culture—negative after last injection. Period of observation—forty-five days after completion of treatment.

(11) Patient, æt. 2 years. History of illness—six months. Spleen—4½″ below costal arch. Temperature—101° to 104°F. Blood culture—positive. W.B.C.—3,000 per c.mm. Urea stibamine, 0·05 grm., 0·1 grm., and 0·2 grm., given intravenously alternately during five days (three injections in all). Temperature came down to normal after first injection. Actual period between first and second injections—two days.

Peripheral blood culture—negative after second injection.

Period of observation—thirty-six days after completion of treatment, during which spleen disappeared below costal arch and W.B.C. count was 10,000 per c.mm.

(12) Patient, æt. 25 years. History of illness—five months. Spleen—6½″ below costal arch. Temperature—apyrexia. Blood culture—positive. W.B.C.—1,800 per c.mm. Urea stibamine, 0·2, 0·3 and 0·35 grm., given intravenously on three successive days (three injections in all). Actual period between first and last injections—two days.

Peripheral blood culture—negative after last injection.

Period of observation—twenty-five days after completion of treatment, during which spleen disappeared below costal arch and W.B.C. count was 9,000 per c.mm.

(13) Patient, æt. 18. History of illness—nine months. Spleen—4″ below costal arch. Jaundice—present. Temperature—99° to 100°F. Blood culture—positive. W.B C.—3,600 per c.mm. Urea stibamine, 0·2, 0·25 and 0·25 grm., given intravenously on alternate days during five days (three injections in all). Temperature came down to normal after first injection. Spleen—disappeared below the costal arch after last injection. Actual period between first and last injections—four days.

Peripheral blood culture—negative after last injection.

Period of observation—twenty-six days after completion of treatment, during which W.B.C. count was 10,400 per c.mm. and jaundice disappeared.

(14) Patient, æt. 25. History of illness—three months. Spleen—5″ below costal arch. Temperature—101° to 103°F. Patient had commencing *cancrum oris*. Blood culture—positive. W.B.C.—1,200 per c.mm. Urea stibamine, 0·1 grm., given intravenously on three successive days (three injections in all). Temperature came

down to normal after first injection. Actual period between first and last injections—two days.

Peripheral blood culture—negative after last injection.

Period of observation—fifteen days after completion of treatment, during which spleen disappeared below costal arch and W.B.C. count was 5,200 per c.mm.

(15) Patient, æt. 35. History of illness—seven months. Spleen —5″ below costal arch. Temperature—100° to 104°F. Blood culture—positive. W.B.C.—1,400 per c.mm. Urea stibamine, 0·1 grm., given intravenously at (1) 8 a.m., (2) 12 a.m., (3) 8 p.m., on 17th May, 1924, and (4) 0·15 grm., at 8 a.m., (5) 0·1 grm., at 12 a.m., (6) 4 p.m., and (7) 8 p.m., on 18th May, 1924 during thirty-six hours (seven injections in all). Temperature came down to normal after first injection.

Actual period between first and last injections—thirty-six hours.

Peripheral blood culture—negative after six injections.

Period of observation—twenty-five days, during which spleen disappeared below costal arch and W.B.C. count was 8,000 per c.mm.

(16) Patient, æt. 28. History of fever—two months. Spleen—4″ below costal arch. Temperature—101° to 104°F. Blood culture—positive. W.B.C.—2,400 per c.mm. Urea stibamine, 0·05 grm., given intravenously at (1) 6 a.m., (2) 0·1 grm., at 10 a.m., and (3) 6 p.m. on 3rd June, 1924, (4) 0·1 grm., at 6 a.m., (5) 10 a.m., (6) 1 p.m. and (7) 6 p.m. on 4th June, 1924, and (8) 0·1 grm., at 12 a.m. and (9) 4 p.m. on 5th June, 1924, during fifty-eight hours (nine injections). Temperature came down to normal after third injection.

Actual period between first and last injections—fifty-eight hours.

Peripheral blood culture—negative after seven injections.

Period of observation—six days, after which the patient refused to remain under further observation.

(17) Patient, æt. 30. History of fever—three months. Spleen —6″ below costal arch. Temperature—99° to 103°F. Blood culture —positive. W.B.C.—2,800 per c.mm. Urea stibamine, 0·05 grm., given intravenously at (1) 6 a.m., 0·1 grm., at (2) 10 a.m. and 0·05 grm., at (3) 4 p.m. on 12th May, 1924, and 0·1 grm., at (4) 6 a.m. (5) 12 a.m. (6) 4 p.m. and (7) 10 p.m. on 13th May, 1924, and 0·1 grm., at (8) 6 a.m. (9) 12 a.m. and (10) 6 p.m. on 14th May, 1924, during sixty hours (ten injections in all). Temperature—normal after ninth injection. Actual period between first and last injections—sixty hours.

Peripheral blood culture—negative after ninth injection.

Period of observation—twelve days, after completion of treatment. Spleen—felt 2″ below the costal arch during the period. W.B.C. count—6,200 per c.mm. Patient refused to remain under further observation.

(18) Patient, æt. 7. History of fever—thirty-two days. Spleen —2½″ below costal arch. Temperature—99° to 100°F. Blood culture—positive. W.B.C.—1,800 per c.mm. Urea stibamine, 0·05 grm., given intravenously at (1) 10 a.m. and 0·1 grm. at (2) 5 p.m. on 3rd May, 1924, 0·1 grm., at (3) 6 p.m. on 4th May, 1924, and 0·15 grm., at (4) 4 p.m. on 5th May, 1924, during fifty-four hours (four injections in all). Temperature—normal after first injection. Actual period between first and last injections—fifty-four hours.

Peripheral blood culture—negative after last injection.

Period of observation—eighteen days, during which spleen disappeared below costal arch and W.B.C. count was 8,800 per c.mm.

(19) Patient, æt. 7 years, History of fever—five months. Spleen—extending up to the umbilicus. Temperature—98° to 102°F. Blood culture—positive. W.B.C.—3,400 per c.mm. Urea stibamine, 0·1 grm., given intravenously at (1) 8 a.m. and (2) 4 p.m. on 20th May, 1924, and 0·1 grm. at (3) 8 a.m. and (4) 4 p.m. on 21st May, 1924, during a period of thirty-two hours (four injections in all). Temperature—normal after second injection. Actual period between first and last injections—thirty-two hours.

Peripheral blood culture—negative after last injection.

Period of observation—thirty-three days after completion of treatment, during which period spleen disappeared below costal arch and W.B.C. count was 11,000 per c.mm.

(20) Patient, æt. 25. History of illness—five months. Spleen —5″ below costal arch. Blood culture—positive. W.B.C.—3,100 per c.mm. Urea stibamine, 0·1 grm., injected intravenously twice in the course of five days. Temperature came down to normal after the first injection. Sterilization proved by complete subsidence of fever, disappearance of the spleen below the costal arch and increase of body weight by 23 lbs. in one and a half month. W.B.C. count rose up to 6,000 per c.mm. during this period. Blood culture—negative one month and a half after treatment was stopped.

(21) Patient, æt. 9 years. History of illness—five months. Spleen—2½″ below costal arch. Liver—slightly enlarged and tender. Temperature—99° to 102°F. Blood culture—positive. W.B.C.—4,200 per c.mm. One injection of 0·05 grm. of urea stibamine and three of 0·1 grm., given intravenously in four injections during twelve days. Temperature—normal after the first injection. Actual period between first and third injections—seven days.

Peripheral blood culture—negative after third injection.

Period of observation—five months after completion of treatment and is still under observation. Patient has markedly improved in health. No fever during five months. W.B.C.—7,500 per c.mm. one month after completion of treatment.

(22) Patient, æt. 10. Spleen—5½″ below costal arch. Temperature—100° to 102°F. Blood culture—positive. W.B.C.—1,800 per c.mm. Urea stibamine, 0·1, 0·15, 0·2, and 0·25 grm., given in six days. Temperature came down to normal after second injection. Actual period between first and last injections—six days.

Peripheral blood culture—negative after last injection.

Period of observation—sixty-five days, during which spleen disappeared below costal arch and W.B.C. count was 8,000 per c.mm.

*N.B.*—Patient had forty-eight injections of sodium antimony tartrate (2·6 grm.) during five months and a half without any benefit. Interval between this treatment and that with urea stibamine—one month and a half.

(23) Patient, æt. 25. Spleen—6″ below costal arch. Temperature—99° to 102°F. Blood culture—positive. W.B.C.—2,600 per c.mm. Urea stibamine, 0·2, 0·25, 0·3, 0·3 and 0·3 grm., given in seven days. Temperature came down to normal after first injection. Actual period between first and last injections—seven days.

Peripheral blood culture—negative after third injection.

Period of observation—forty days, during which spleen disappeared below the costal arch and W.B.C. count was 6,800 per c. mm.

*N.B.*—Patient had a course of fifty-six injections of sodium antimony tartrate during six months and a half and second course of treatment of twenty-four injections of the same and twenty-two

injections of tartar emetic alternately during seven months. Interval between this treatment and that with urea stibamine—four months.

(24) Patient, æt. 3. Spleen—4½″ below costal arch. Temperature—100° to 104°F. Blood culture—positive. W.B.C.—2,600 per c.mm. Urea stibamine, 0·05 grm.. 0·1 grm., 0·15 grm., 0·2 grm., and 0·2 grm., given intravenously in five injections during nine days. Temperature came down to normal after first injection. Actual period between first and fourth injections—six days.

Peripheral blood culture—negative after fourth injection.

Period of observation—five months. Spleen disappeared below costal arch in two months and W.B.C. count was 8,800 per c.mm. at the end of four months.

*N.B.*—Patient had forty-five injections of sodium antimony tartrate during five months without any benefit. Interval between this treatment and that with urea stibamine—three months.

(25) Patient, æt. 25. Spleen—6″ below costal arch. Temperature—98° to 102°F. Blood culture—positive. W.B.C.—1,200 per c.mm. Urea stibamine, 0·25, 0·25, 0·3, 0·3, 0·3, 0·3 and 0·3 grm., given intravenously in seven injections during twelve days. Temperature—normal after first injection. Actual period between first and fifth injections—seven days.

Peripheral blood culture—negative after fifth injection.

Period of observation—fifty-five days, during which spleen disappeared below costal arch and W.B.C. count was 7,000 per c. mm.

*N.B.*—Patient had two courses of treatment. During his first course, he had thirty-six injections of sodium antimonyl tartrate (3 grms.) and twenty injections of tartar emetic (1·2 grms.) during five months and a half. Second course of treatment consisting of fifteen injections of sodium antimonyl tartrate (1·2 grms.) and twelve injections of tartar emetic (1 grm.) during two months. Altogether 6·4 grms. Interval between the two courses of treatment and that with urea stibamine—five months.

(26) Patient, æt. 20. Spleen—3″ below costal arch. Temperature—99°F. Blood culture—positive. W.B.C.—2,800 per c.mm.

Urea stibamine, 0·1, 0·1, 0·2 and 0·2 grm., given intravenously in four injections during five days. Temperature came down to normal after first injection and spleen could not be felt below the costal arch after third injection. Actual period between first and last injections—five days.

Peripheral blood culture—negative after last injection.

Period of observation—five months and a half, during which spleen disappeared below costal arch and W.B.C. count was 7,800 per c.mm.

*N.B.*—Patient had thirty-three injections of sodium antimonyl tartrate during four months without any benefit. Interval between this treatment and that with urea stibamine—forty-eight days.

(27) Patient, æt. 22. Spleen—4½″ below costal arch. Temperature—99° to 103°F. Blood culture—positive. W.B.C.—1,000 per c.mm. Urea stibamine, 0·2, 0·2, 0·25, 0·25 and 0·25 grm., given intravenously in five injections during eight days. Temperature came down to normal after second injection. Actual period between first and third injections—four days.

Peripheral blood culture—negative after three injections.

Period of observation—three months, during which spleen disappeared below costal arch and W.B.C. count was 6,800 per c.mm.

*N.B.*—Patient had thirty-two injections of sodium antimonyl tartrate (2·2 grms.) during four months without any benefit. Interval between this treatment and that with urea stibamine—two months and a half.

## Observations

(1) The present paper gives a number of cases of kala-azar collected from the observations of different observers in which cure was brought about in 32 hours to seven days after treatment with urea stibamine.

(2) The intensive method, adopted by us in a few cases, of giving multiple injections on the same day so as to maintain a constant high concentration of urea stibamine in the

blood has led to the remarkable shortening of the period required for sterilization of the peripheral blood. Though no untoward results were met with in these cases, yet it is too early to state whether the method may be universally advocated.

(3) The mechanism of response of *Leishmania* to an antimonial preparation is a very complicated one. While it is universally admitted that urea stibamine brings about sterilization of an infected individual in a much shorter time than tartar emetic or sodium antimonyl tartrate, it is at the same time observed that even with urea stibamine the time required for sterilization is variable. In the present series of cases, this was brought about in seven or less than seven days and in a few cases in thirty-two hours. Though, in some of the cases, striking results were obtained by the intensive method adopted by us in some of our cases, yet there is no doubt that, apart from this, some cases are more quickly amenable to treatment than others. What is the mechanism of this variability? This constitutes an important line of research.

(4) The percentage of cases very easily amenable to urea stibamine in a series of unselected cases will be most interesting.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, February 18, 1925]

## PART XV

### FURTHER OBSERVATIONS ON CERTAIN DERIVATIVES OF *p*-AMINO-PHENYL-STIBINIC ACID

The present paper is a continuation of a previous one published in the *Indian Journal of Medical Research*, October, 1922, and a second one published in the same Journal, October, 1924.

The preparation of the sodium salt of *p*-amino-phenyl-stibinic acid, which was named stibamine (Brahmachari), has been described in the above-mentioned papers.

### (1) 2-CHLORO-1-ACETYL-AMINO-PHENYL-4-SODIUM STIBINATE. $C_8H_8O_4NCl\ Sb\ Na$

For the sake of simplicity, we shall name this salt chloro-stibacetin.

Ten grammes of acetyl-*p*-amino-phenyl-stibinate of sodium are dissolved in 500 c.c. of water. The solution is cooled with ice and 140 c.c. of sodium hypochlorite solution (=2 grms. of Cl) added to it with vigorous stirring for half an hour. Then dilute acetic acid is added to the above in excess. The whole mixture is vigorously stirred in the cold and filtered after one hour. The precipitate is collected and dissolved in excess of ammonia and filtered. To the filtrate is added dilute acetic acid and the precipitate formed is

filtered and washed with distilled water. It is dissolved in caustic soda solution and neutralized with dilute acetic acid and filtered. After concentrating the filtrate, chloro-stibacetin is precipitated by absolute alcohol and dried *in vacuo* over fused calcium chloride.

The above compound has the same composition as that of von Heyden's '471'—Stibosan.

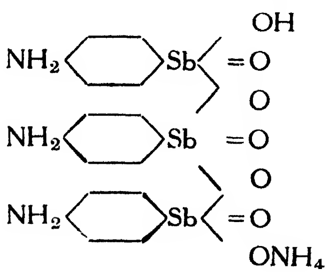
### (2) AMMONIUM SALT OF *p*-AMINO-PHENYL STIBINIC ACID. $C_{18}H_{23}O_7N_4Sb_3$

For the sake of simplicity, we shall name this salt ammonium stibamine.

*Preparation*

*p*-amino-phenyl-stibinic acid is dissolved in the least quantity of ammonia in the cold. The acid is reprecipitated by addition of dilute acetic acid. It is filtered and the precipitate is washed several times with distilled water. The precipitate is then dissolved in the least quantity of ammonia and the solution carefully neutralized with dilute acetic acid. It is again filtered and the filtrate is concentrated and then absolute alcohol added to the solution. Ammonium stibamine is precipitated and filtered and subsequently dried *in vacuo* over fused calcium chloride.

It may be noted that in the formation of the ammonium salt *p*-amino-phenyl-stibinic acid polymerizes with the formation of a compound of the following type :



The antimony content of the above compound is high and therefore it should be of great therapeutic value in *leishmaniasis*.

The same peculiarity is observed in the case of ammonium stibamine as in the case of stibamine, that when a free amino-group is present in the benzene nucleus of aromatic amino-stibinic acids, it polymerizes during salt formation, three molecules of the acid polymerizing with separation of two molecules of water.

The toxicity of the above compounds will form the subject of another communication.

We have observed that glucose has the property of combining with the amino-group of *p*-amino-phenyl stibinic acid and its derivatives, just as it has the property of combining with salvarsan.

### (3) COMBINATION OF GLUCOSE WITH STIBAMINE

We shall name this compound glucose-stibamine.

#### *Preparation*

Eight grammes of stibamine are dissolved in 40 c.c. of water containing 6 grms. of glucose. The solution is then heated to 60° to 65°C. for nearly two hours. It is then filtered and the filtrate concentrated. Absolute alcohol is then added slowly to the filtrate. The precipitate obtained is collected and washed twice with a mixture of alcohol (4 to 1) and subsequently with absolute alcohol. It is subsequently dried over calcium chloride *in vacuo*

Glucose-stibamine is a yellowish powder, very easily soluble in water.

### (4) COMBINATION OF GLUCOSE WITH AMMONIUM STIBAMINE

We shall name this compound glucose-ammonium stibamine.

*Preparation*

This is prepared in the same way as the above by using ammonium stibamine in place of stibamine. It is less soluble in water than glucose-stibamine.

Detailed observations on these and other allied glucose compounds will be communicated in a subsequent series.

## (5) COMBINATION OF GLUCOSE WITH UREA STIBAMINE AND STIBGLYCINE AMIDE

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, February 18, 1925]

## PART XVI

## OBSERVATIONS ON BLOOD-CULTURE OF KALA-AZAR PATIENTS ON N.N.N. MEDIUM DURING 1922-24

I. COMPARATIVE VALUE OF PERIPHERAL BLOOD CULTURE, SPLEEN BLOOD CULTURE AND SPLEEN PUNCTURE IN THE DIAGNOSIS OF KALA-AZAR

II. THE PERIOD AT WHICH STERILIZATION OF THE PERIPHERAL BLOOD TAKES PLACE DURING TREATMENT WITH UREA STIBAMINE

### I. Comparative Value of Peripheral Blood Culture, Spleen Blood Culture and Spleen Puncture in the Diagnosis of Kala-azar

(1) In a series of 440 positive cases, 426 showed positive peripheral blood culture, *i.e.*, 97 per cent. [These positive cases include (*a*) 440 cases in which peripheral blood culture was made and found positive in 426, and (*b*) 190 cases in which splenic culture was made and found positive in all.]

(2) In a series of 220 positive cases, 186 showed positive spleen puncture results, *i.e.*, 84·5 per cent.

[These positive cases include (*a*) 160 cases in which peripheral blood culture was made and found positive in 154 cases, and (*b*) 170 cases in which spleen blood culture was made and found positive in all.]

## II. The Period at which Sterilization of the Peripheral Blood Takes Place during Treatment with Urea Stibamine

*The following table shows the period in which sterilization took place during treatment with urea stibamine*

| Serial No. | Age. / Duration of illness. | Number of injections and total amount of urea stibamine after which blood culture became negative. / Period during which the total amount of urea stibamine was given. | Period after commencement of treatment during which blood culture remained positive. | Period after commencement of treatment at which blood culture became negative. | Result of blood culture during period of observation. | Number of times blood culture was made. During treatment. | During period of observation. |
|---|---|---|---|---|---|---|---|
| 1 | 14 mths / 3 mths. | 2—0·15 grm. / 72 hrs. | 76 hrs. | 80 hrs. | Negative (45 days) | 4 | 3 |
| 2 | 2 yrs. / 6 mths. | 2—0·15 grm. / 48 hrs. | 54 hrs. | 58 hrs | Negative (36 days) | 4 | 4 |
| 3 | 7 yrs. / 32 days | 4—0·4 grm. / 53 hrs. | 62 hrs. | 70 hrs. | Negative (18 days) | 9 | 3 |
| 4 | 7 yrs. / 5 mths. | 4—0·35 grm. / 32 hrs. | 40 hrs. | 48 hrs. | Negative (28 days) | 5 | 6 |
| 5 | 25 yrs. / 5 mths. | 3—0·85 grm. / 72 hrs. | 78 hrs. | 84 hrs. | Negative (25 days) | 6 | 6 |
| 6 | 18 yrs. / 9 mths | 3—0·7 grm. / 144 hrs. | 148 hrs. | 152 hrs. | Negative (26 days) | 10 | 5 |
| 7 | 25 yrs. / 3 mths. | 3—0·3 grm. / 72 hrs. | 76 hrs. | 78 hrs. | Negative (15 days) | 8 | 6 |
| 8 | 20 yrs. / 4 mths. | 3—0·4 grm. / 120 hrs. | 120 hrs. | 124 hrs. | Negative (25 days | 6 | 7 |
| 9 | 35 yrs. / 9 mths. | 6—0·65 grm. / 32 hrs. | 32 hrs. | 35 hrs. | Negative (28 days) | 6 | 14 |
| 10 | 28 yrs. / 2 mths. | 6—0·6 grm. / 31 hrs. | 36 hrs. | 49 hrs. | Negative (6 days) | 5 | 3 |

| Serial No. | AGE. / Duration of illness. | NUMBER OF INJECTIONS AND TOTAL AMOUNT OF UREA STIBAMINE AFTER WHICH BLOOD CULTURE BECAME NEGATIVE. / Period during which the total amount of urea stibamine was given. | Period after commencement of treatment during which blood culture remained positive. | Period after commencement of treatment at which blood culture became negative. | Result of blood culture during period of observation. | NUMBER OF TIMES BLOOD CULTURE WAS MADE. During treatment. | During period of observation. |
|---|---|---|---|---|---|---|---|
| 11 | 30 yrs. / 3 mths. | 9–0·88 grm. / 54 hrs. | 52 hrs. | 60 hrs. | Negative (10 days) | 4 | 2 |
| 12 | 10 yrs. / 6 mths. | 3–0·75 grm. / 120 hrs. | 122 hrs. | 126 hrs. | Negative (65 days) | 6 | 6 |
| 13 | 25 yrs. / 6 mths. | 3–0·75 grm. / 96 hrs. | 106 hrs. | 120 hrs | Negative (40 days) | 8 | 6 |
| 14 | 3 yrs. / 4 mths. | 4–0·35 grm. / 144 hrs. | 148 hrs. | 155 hrs. | Negative (150 days) | 9 | 17 |
| 15 | 24 yrs / 6 mths. | 5–1·4 grms. / 108 hrs. | 168 hrs. | 172 hrs. | Negative (51 days) | 13 | 18 |
| 16 | 20 yrs. / 4 mths. | 4–0·6 grm. / 120 hrs. | 168 hrs. | 192 hrs. | Negative (165 days) | 15 | 13 |
| 17 | 22 yrs. / 2 mths. | 5–1·15 grms. / 168 hrs. | 204 hrs. | 216 hrs. | Negative (90 days) | 14 | 16 |
| 18 | 16 yrs. / 6 mths. | 6–0·6 grm. / 240 hrs. | 288 hrs. | 312 hrs. | Negative (65 days) | 11 | 16 |
| 19 | 9 yrs. / 6 mths. | 3–0·15 grm. / 168 hrs. | 216 hrs. | 240 hrs. | Negative (90 days) | 7 | 4 |
| 20 | 20 yrs. / 9 mths. | 6–0·8 grm. / 240 hrs. | 264 hrs. | 288 hrs. | Negative (34 days) | 12 | 6 |
| 21 | 30 yrs. / 6 mths. | 6–0·8 grm. / 192 hrs. | 226 hrs. | 240 hrs. | Negative (36 days) | 13 | 12 |
| 22 | 12 yrs. / ? mths | 6–0·6 grm. / 230 hrs. | 234 hrs. | 240 hrs | Negative (26 days) | 12 | 8 |
| 23 | 26 yrs. / 11 mths. | 6–0·8 grm. / 192 hrs. | 204 hrs. | 216 hrs | Negative (65 days) | 10 | 9 |
| 24 | 20 yrs. / 5 mths. | 5–0·8 grm. / 168 hrs. | 180 hrs. | 192 hrs. | Negative (129 days) | 8 | 12 |

| Serial No. | AGE. / Duration of illness. | NUMBER OF INJECTIONS AND TOTAL AMOUNT OF UREA STIBAMINE AFTER WHICH BLOOD CULTURE BECAME NEGATIVE. / Period during which the total amount of urea stibamine was given. | Period after commencement of treatment during which blood culture remained positive. | Period after commencement of treatment at which blood culture became negative. | Result of blood culture during period of observation. | NUMBER OF TIMES BLOOD CULTURE WAS MADE. During treatment. | During period of observation. |
|---|---|---|---|---|---|---|---|
| 25 | 21 yrs. / 3 mths. | 5–0·8 grm. / 14 days | 14 days | 15 days | Negative (30 days) | 9 | 10 |
| 26 | 8 yrs. / 11 mths | 4–0·55 grm. / 264 hrs. | 300 hrs. | 312 hrs. | Negative (60 days) | 21 | 14 |
| 27 | 11 yrs. / 6 mths. | 4–0·8 grm. / 264 hrs. | 276 hrs. | 288 hrs. | Negative (92 days) | 14 | 23 |
| 28 | 18 yrs. / 5 mths. | 3–0·4 grm. / 168 hrs. | 192 hrs | 216 hrs. | Negative (75 days) | 9 | 12 |
| 29 | 9 yrs. / 4 mths. | 4–0·4 grm. / 264 hrs. | 312 hrs. | 336 hrs. | Negative (65 days) | 13 | 10 |
| 30 | 28 yrs. / 4 mths. | 9–1·8 grms. / 360 hrs. | 384 hrs. | 408 hrs. | Negative (60 days) | 17 | 12 |
| 31 | 25 yrs. / 5 mths. | 5–1 grm. / 192 hrs. | 192 hrs. | 216 hrs | Negative (72 days) | 8 | 12 |
| 32 | 10 yrs. / 6 mths. | 6–0·6 grm. / 192 hrs. | 204 hrs. | 216 hrs. | Negative (90 days) | 12 | 14 |
| 33 | 25 yrs. / 5 mths. | 6–1·2 grms. / 296 hrs. | 308 hrs. | 320 hrs. | Negative (30 days) | 15 | 14 |
| 34 | 8 yrs. / 1 yr. | 4–0·8 grm. / 144 hrs. | 216 hrs. | 240 hrs. | Negative (60 days) | 10 | 10 |
| 35 | 16 yrs. / 3 mths. | 5–0·8 grm. / 168 hrs. | 180 hrs. | 192 hrs. | Negative (40 days) | 10 | 9 |
| 36 | 26 yrs. / 5 mths. | 3–0·85 grm. / 17 days | 17 days and 12 hrs. | 18 days | Negative (45 days) | 15 | 11 |
| 37 | 42 yrs. / 9 mths. | 4–1·15 grms. / 28 days | 32 days | 34 days | Negative (56 days | 14 | 12 |

Making a summary of the above cases, we can divide them into the following groups :—

| | |
|---|---|
| (1) Number of cases in which sterilization took place in from 32 hours to 84 hours (less than four days) ... ... ... ... | 9 |
| (2) Number of cases in which sterilization took place in from above 84 hours to 144 hours (6 days) ... | 3 |
| (3) Number of cases in which sterilization took place in from above 144 hours to 240 hours (10 days) ... ... ... | 15 |
| (4) Number of cases in which sterilization took place in above 10 days to 16 days ... ... | 7 |
| (5) Number of cases in which sterilization took place in more than 16 days ... ... ... | 2 |
| (6) One case required 34 days for sterilization ... | 1 |

From the above we conclude—

26 out of 37 cases, *i.e.*, 73 per cent of the cases were sterilized within 10 days after commencement of treatment with urea stibamine.

[Reprinted from the Indian Journal of Medical Research, Vol. XIII, No. 1, July, 1925]

# CHEMOTHERAPHY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, March 19, 1925]

## PART XVII

### FURTHER DETAILS OF THE PREPARATION OF UREA STIBAMINE

In the *Indian Journal of Medical Research* (October, 1922), I described the method of preparing urea stibamine. The details of preparing acetyl-*p*-amino-phenyl-stibinic acid and *p*-amino-phenyl-stibinic acid which are two substances required in the preparation of urea stibamine, not having been described in the above paper, are described below :—

*(1) Preparation of acetyl-p-amino-phenyl-stibinic acid*

One gram-molecule of para-amino-acetanilide is added to well-cooled sulphuric acid (1·5 gram-molecule) in one litre of water. The mixture is then diazotised with a cooled solution of sodium nitrite (one gram-molecule) in water. The solution is then added to a solution of sodium antimonite which is rapidly cooled to 0°C. [The sodium antimonite solution is prepared as follows: 600 grammes of sodium hydroxide are dissolved in 3 litres of water. The solution is added to aqueous antimony trichloride prepared by dissolving antimony trioxide (·5 gram-molecule) in 764 grammes of hydrochloric acid (D 1·123).] After the reaction is complete, the solution is almost neutralised with dilute sulphuric acid and the remainder of the caustic alkali is removed by saturating the solution with carbon dioxide. The solution

is now filtered and the filtrate is saturated with sodium chloride when acetyl-*p*-amino-phenyl-stibinate of sodium is precipitated. The precipitate is then dissolved in water and again saturated with carbon dioxide. The solution is filtered and the filtrate treated with dilute hydrochloric acid which precipitates acetyl-*p*-amino-phenyl-stibinic acid.

(2) *Preparation of p-amino-phenyl-stibinic acid*

One part of acetyl-*p*-amino-phenyl-stibinic acid is heated for some hours with 10 parts of 5 per cent aqueous sodium hydroxide for some hours until the dilute sample gives with dilute hydrochloric acid a precipitate which dissolves in excess of the acid. To the cooled solution is added dilute acetic acid when *p*-amino-phenyl-stibinic acid is precipitated.

The *p*-amino-phenyl stibinic acid is then suspended in water and urea added to the suspension till the whole of the acid is almost dissolved. The solution is facilitated by gentle heating. It is then filtered and the filtrate concentrated on the water bath. The concentrated solution after cooling is mixed with excess of alcohol which precipitates urea stibamine. The precipitate obtained is further washed with alcohol to free it from any uncombined urea. It is subsequently dried over a porous plate.

The constitution of urea stibamine has been fully discussed by myself and my chemist Mr. Judhisthir Das, in the *Indian Journal of Medical Research* (October, 1924).

*Repinted from the Indian Journal of Medical Research, Vol. XIII, No. 3, January, 1929*

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

[Received for Publication, September 23, 1925]

## PART XVIII

### FURTHER OBSERVATIONS ON CERTAIN DERIVATIVES OF *p*-AMINO-PHENYL-STIBINIC ACID *(Continued)*

This paper is a continuation of the paper entitled *Chemotherapy of Antimonial Compounds in Kala-azar Infection, Part XV*, published in the *Indian Journal of Medical Research*, July, 1925.

### (6) GLUCOSE COMPOUNDS WITH *p*-AMINO-PHENYL-STIBINIC ACID AND ITS DERIVATIVES

These, already referred to in our paper (July, 1925), consist of glucose compounds with (1) stibamine, (2) ammonium stibamine, (3) urea stibamine, and (4) stibglycine amide.

### (7) CONDENSATION OF DICHLORO-ACETAMIDE WITH *p*-AMINO-PHENYL-STIBINIC ACID

#### *Experimental*

Four grammes of *p*-amino-phenyl-stibinic acid are treated wtih a watery solution of caustic soda till it dis-

solves. The solution is made slightly alkaline with a slight excess of caustic soda. To the solution one gramme of dichloro-acetamide is added and the resulting mixture is heated at 60° to 70°C, the solution being kept slightly alkaline by addition of small quantities of caustic soda, from time to time. It is then filtered and the filtrate cooled in ice. Dilute hydrochloric acid is now added in excess to the solution and the precipitate obtained is washed twice in dilute hydrochloric acid and subsequently five times in distilled water. The precipitate is then dissolved in caustic soda and the solution subsequently made neutral. This solution is filtered and concentrated, and to the concentrated solution absolute alcohol is added in excess. The precipitate obtained is filtered and washed in absolute alcohol and dried over calcium chloride in a vacuum desiccator.

### (8) THE SODIUM SALT OBTAINED BY THE REPLACEMENT OF $NH_4$ FROM UREA STIBAMINE BY Na

This has already been described in *Indian Journal of Medical Research*, October, 1924. It is sodium-carbamino-*p*-stibanilate. Its glucose compound has also been prepared.

The glucose compounds described in the present paper are prepared in the same way as the glucose compounds with stibamine and ammonium stibamine (*Indian Journal of Medical Research*, July, 1925).

Therapeutically, the glucose compounds are weaker than the corresponding antimony compounds from which they are derived.

[*Reprinted from the Calcutta Medical Journal, June, 1926*, and *the Indian Journal of Medicine, June, 1926*]

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

**(New Series)**

## PART I

### THERAPEUTIC VALUE OF N-PHENYL-GLYCINE-AMIDE-*p*-STIBINATE OF SODIUM

( A PRELIMINARY NOTE )

The following is a note of the first series of cases of kala-azar treated with N-phenyl-glycine-amide-*p*-stibinate of sodium by Brahmachari and co-worker.

1. Patient named D., æt. 16, was admitted into Brahmachari's ward, with history of fever for 6 months. On admission, his spleen extended five inches below the costal arch. Blood culture on NNN medium—positive flagellate culture. Patient was treated with intravenous injection of the above compound. Altogether 20 injections were given. As a result of treatment the fever completely stopped, the spleen could not be felt below the costal arch, and at the time of discharge blood culture on NNN medium was negative.

Dose—·05 to ·20 gramme. Increase of body weight after treatment—1st. 1lb.

*Result of Blood Examination*—

(1) R. B. C.—1,800,000, W. B. C.—2,500, Hb.—35% on 11-2-25 before treatment.

(2) R. B. C.—4.500,000, W. B. C.—8,400, Hb.—85% on 20-6-25 after treatment.

2. Patient named J., æt. 10, was admitted into Brahmachari's ward on 17-11-24, with history of fever for five months. On admission, his spleen extended four inches and a quarter below the costal arch. Patient was treated with intravenous injection of the above compound. Altogether 15 injections were given. The fever stopped after the 3rd injection.

Dose—·05 to ·20 gramme. Increase in body weight after treatment—1 stone.

*Result of Blood Examination*—

(1) R. B. C.—2,500,000, W. B. C.—3,500, Hb.—45% on 19-11-24 before treatment.

(2) R. B. C.—4,400,000, W. B. C.—9,600, Hb.—45% on 27-3-25 after treatment.

3. Patient named S., æt. 14, was admitted into Brahmachari's ward on 3-5-25, with history of fever for three months. On admission, his spleen extended 2½ inches and the liver 1 inch below the costal arch. Patient was treated with intravenons injection of the above compound. Altogether 12 injections were given. Fever stopped after the 4th injection.

Dose—·05 to ·20 gramme.

*Result of Blood Examination*—

(1) R. B. C.—2,000,000, W. B. C.—1,800, Hb.—40% on 20-6-25 before treatment.

(2) R. B. C.—4,200,000, W. B. C.—7,100, Hb.—70% on 12-8-25 after treatment.

4. Patient named J., æt. 10, was admitted into Brahmachari's ward on 2-3-1925, with history of fever for four months. On admission, his spleen extended up to

the umbilicus and the liver was just palpable below the costal arch. Patient was treated with intravenous injection of the above compound. Altogether 10 injections were given. The temperature came down to normal after the 4th injection. At the time of discharge, the spleen and the liver could not be felt below the costal arch.

Dose—·05 to ·20 gramme.

*Result of Blood Examination*—

(1) R. B. C.—2,500,000, W. B. C.—2,500, Hb.—45% on 8-3-25 before treatment.

(2) R. B. C.—4,200,000, W. B. C.—6,800, Hb.—80% on 10-6-25 after treatment.

5. Patient named S., æt. 13, was admitted into Brahmachari's ward on 17-3-1925, with history of fever for twelve months. On admission, his spleen extended 2 inches below the costal arch. The patient was treated with intravenous injection of the above compound. Altogether 20 injections were given. The temperature came down to normal after the 4th injection. At the time of discharge the spleen could not be felt below the costal arch.

Dose—·05 to ·20 gramme. Increase in body weight after treatment—1 st. 1 lb.

*Result of Blood Examination*—

(1) R. B. C.—3,500,000, W. B. C.—3,100, Hb.—65% on 18-3-25 before treatment.

(2) R. B. C.—4,000,000, W. B. C.—5,100, Hb.—75% on 10-5-25 after treatment.

6. Patient named M., æt., 20, was admitted into Brahmachari's ward on 25-5-25, with history of fever for two years. On admission, his spleen extended 6 inches below the costal arch. Patient was treated with intravenous injection of the above compound. Altogether 15 injections

were given. The temperature came down to normal after the 5th injection. At the time of discharge the spleen was felt 2 inches below the costal arch.

Dose—·05 to ·20 gramme.

*Result of Blood Examination*—

(1) R. B. C.—2,500,000, W. B. C.—2,100, Hb.—45% on 27-5-25 before treatment.

(2) R. B. C.—3,200,000, W. B. C.—6,500, Hb.—70% on 17-8-25 after treatment.

7. Patient named S., æt. 21, was admitted into Brahmachari's ward on 28-2-1925. On admission, both his spleen and the liver extended 4 inches below the costal arch. Patient was treated with intravenous injection of the above compound. Altogether 14 injections were given. The temperature came down to normal after the 5th injection. At the time of discharge the spleen was felt 1 inch below the costal arch.

Dose—·05 to ·20 gramme.

*Result of Blood Examination*—

(1) R. B. C.—2,820,000, W. B. C.—1,800, Hb.—30% on 31-3-25 before treatment.

(2) R. B. C.—3,000,000, W. B. C.—5,000, Hb.—65% on 29-5-25 after treatment.

8. Patient named B., æt. 12, was admitted into Brahmachari's ward on 23-3-1925. On admission his spleen extended 8 inches and his liver 2 inches below the costal arch. Patient was jaundiced. He was treated with intravenous injection of the above compound. Altogether 10 injections were given. The temperature came down to normal after the 6th injection. At the time of discharge the spleen could not be felt below the costal arch.

Dose—·05 to ·20 gramme. Increase of weight after treatment—1 st. 7 lbs.

*Result of Blood Examination*—

(1) R. B. C.—2,500,000, W. B. C.—2,800, Hb.—20% on 26-3-25 before treatment.

(2) R. B. C.—4,500,000, W. B. C.—6,200, Hb.—80% on 10-6-25 after treatment.

## Remarks

This paper gives records of preliminary observations made by Brahmachari on the treatment of kala-azar with N-phenyl-glycine-amide-*p*-stibinate of sodium. In this paper it is not possible to make any comparison of its value with that of other aromatic antimonials. Further investigation is in progress.

*[Reprinted from the Calcutta Medical Journal, August, 1926, and the Indian Journal of Medicine, September, 1926]*

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

**(New Series)**

## PART II

### THE TOXICITY AND THERAPEUTIC VALUE OF OLD SAMPLES OF UREA STIBAMINE

Samples of urea stibamine kept for indefinite periods in sealed ampoules under ordinary conditions in India have been tested to determine whether they differed in their toxicity and therapeutic value when compared with fresh samples. The samples tested were those that had been kept in sealed ampoules from the beginning of the year 1922. Their toxicity was tested in January, 1926.

*Lethal Effects Produced from the Administration of a 2 per cent Solution of Urea Stibamine to Guinea-pigs by Intramuscular Injection*

*Toxicity of fresh samples of urea stibamine*

| Dose in gram. per kilo. of body weight. | Number of guinea-pigs used. | Number of guinea-pigs died. | |
|---|---|---|---|
| ·70 | 4 | 4 | Minimum lethal dose. |
| ·65 | 3 | 2 | |
| ·60 | 4 | 2 | |
| ·50 | 2 | 1 | |
| ·45 | 4 | 1 | |
| ·45 | 4 | 1 | |
| ·35 | 6 | *nil* | Maximum tolerated dose. |

*Toxicity of old samples of urea stibamine*

| Dose in gram. per kilo. of body weight. | Number of guinea-pigs used | Number of guinea-pigs died. | |
|---|---|---|---|
| ·70 | 6 | 6 | Minimum lethal dose. |
| ·65 | 3 | 2 | |
| ·60 | 3 | 2 | |
| ·45 | 3 | 1 | |
| ·40 | 3 | 1 | |
| ·35 | 6 | *nil* | Maximum tolerated dose. |

It will be seen that there is no difference between old and new samples of urea stibamine in their toxicity as tested on guinea-pigs.

### *Physical and Chemical Properties*

No difference was observed in the physical and chemical properties between old and new samples of urea stibamine kept in sealed ampoules.

### *Therapeutic Properties*

No difference was observed in the therapeutic value of old samples of urea stibamine as compared with new ones. No untoward symptoms were met with during the course of treatment with these samples. Their therapeutic value was tested in three cases. One of them was a case suffering from acute kala-azar with high fever and delirium and other severe constitutional symptoms. Blood culture for flagellates was positive. The temperature came down to normal after three injections of an old sample of urea stibamine kept in sealed tubes since 1922. After 8 injections were given blood culture was found to be negative and then the injections were stopped. The second case recovered after 10 and the third after 12 injections of the same samples of urea stibamine.

[Reprinted from the *Calcutta Medical Journal, February*, 1927, and *Indian Journal of Medicine, February, 1927*]

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

## (New Series)

## PART III

### DERMAL LEISHMANOID WITH POSITIVE FLAGELLATE CULTURE FROM THE PERIPHERAL BLOOD

Under the name of dermal leishmanoid a rare form of skin lesion was originally described by Brahmachari in 1923, which was characterised by a multiple infection of the skin by *Leishmania-donovani* in individuals who had previously suffered from kala-azar (visceral leishmaniasis) and were subsequently cured by a course of antimonial treatment. This observation has since been confirmed (Brahmachari, Megaw, Knowles, Acton and others).

Very rarely cases may be observed in which similar skin lesions due to leishmania-donovani infection may be found developing in an imperfectly cured case of kala-azar and in which the skin lesions more or less persist after sterilization of the internal organs against leishmania by a course of antimonial treatment.

The following is one such extremely rare case and is therefore of exceptional interest.

Patient, named G. A., Mahomedan, was at first treated for kala-azar by a course of treatment with sodium antimonyl tartrate. Six months later, he was admitted into hospital with nodular eruptions all over the body. He stayed in hospital for nearly three weeks during which he was treated with 5 injections of Von Heyden's 471 (stibosan) without

any improvement. He had also had treatment with galvanic current exposures in the Calcutta School of Tropical Medicine without any benefit. Patient came under our observation on 23rd July, 1926, about six months after he had left treatment.

*Condition at the time of our first observation*—Patient had very well marked nodular eruptions over his face, the trunk and the extremities (see Plate). Besides these, there were patches of a-pigmentation over the body especially the trunk. He was suffering from fever with enlargement of the spleen.

Blood examination on 28th July, 1926, before treatment with urea stibamine (Brahmachari) was as follows :—

| | | |
|---|---|---|
| R. B. C.—4,000,000. | Leucocyte count— | |
| W. B. C.—2,700. | Polymorphonuclears | ... 61% |
| Hb.—75% | Mononuclears | ... 18% |
| | Lymphocytes | ... 17% |
| | Eosinophiles | ... 4% |

No malarial parasites. Culture from the peripheral blood on Kligglers' media gave positive flagellate culture. Wasserman reaction—negative. The nodules in the skin showed *Leishmania-donovani* on smear and culture.

Patient was put on a course of treatment with urea stibamine (Brahmachari) from 15th August, 1926. The spleen quickly diminished in size and the fever stopped. The skin eruptions seemed to be very resistant to treatment, though they slowly diminished from the trunk and extremities after a course of treatment of bi-weekly intravenous injections with urea stibamine extending over a period of five months.

Blood examination on 13th September, 1926, after treatment with urea stibamine (Brahmachari) :—

| | | |
|---|---|---|
| R. B. C.—4,500,000. | Leucocyte count— | |
| W. B. C.—6,600. | Polymorphonuclears | ... 61% |
| Hb.—75% | Mononuclears | ... 18% |
| | Lymphocytes | ... 19% |
| | Eosinophiles | ... 2% |

Blood culture for flagellates—negative.

## Observations

The present case is of exceptional interest for the following reasons :—

(1) The patient developed "dermal leishmanoid" at a stage when he was not completely cured of kala-azar as shown by positive flagellate culture from the peripheral blood, leucopenia with fever and enlargement of the spleen. Though positive flagellate culture from the peripheral blood could be due to the blood having been accidentally infected from the skin at the time of drawing the blood, yet the clinical picture of the case at the time when it came under observation, showed that the patient was not cured of kala-azar.

(2) He was subsequently cured of kala-azar after a course of treatment with urea stibamine (Brahmachari).

(3) The skin eruptions are very resistant to treatment with antimony as they seemed to be only very slowly yielding to treatment with urea stibamine.

The case appears to have been one of incompletely cured kala-azar at the time of our first observation and was met with at a stage when visceral leishmaniasis was passing into dermal leishmanoid. It is therefore the first of its kind, as, up to now, all cases of dermal leishmanoid that have been recorded were found to have developed in kala-azar patients after a definite period of recovery from visceral leishmaniasis.

[*Reprinted from the Indian Medical Gazette, Vol. LXIII, No. 7, July, 1928,* and *the Indian Journal of Medicine, Vol. X, Part IV, August, 1929, with slight modification, by kind permission of the Editor, Indian Medical Gazette*]

# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS IN KALA-AZAR INFECTION

## (New Series)

## PART IV

### A RARE CASE OF DERMAL LEISHMANOID *

After attention was drawn by Brahmachari to skin eruptions due to infection with leishmania-donovani, Acton in collaboration with Napier has described the following stages of this disease :—

(1) An early de-pigmented stage.

(2) A later nodular stage.

(3) A xanthoma-type of the disease in which there is a tendency towards fibrous tissue formation, and constriction of venules and subsequent dilatation.

It is stated by these authors that the nodules appear in the place of de-pigmented patches.

Generally speaking, cases of dermal leishmanoid come for treatment when both the nodules and de-pigmented patches are present in the skin. The present case is therefore of much interest, as it showed an erythematous patch over the face and a few nodules over it with complete

* Read at a meeting of the Medical Section of the Asiatic Society of Bengal, April 16, 1928.

absence of de-pigmentation in any part of the body, confirming the view of Brahmachari that the disease may first show itself in the form of patches of erythema over the skin before the appearance of de-pigmentation or nodules.

*History—*

About three years ago the patient had an attack of kala-azar. He was treated with about thirty injections of sodium antimonyl tartrate and was apparently cured. About a year ago he noticed a small patch over the bridge of the nose which gradually extended and assumed the present size. A few papules appeared subsequently over the erythematous area.

*Present Condition—*

Patient is a healthy individual. There is no enlargement of spleen or liver. There is no fever. Blood culture is negative. There is no anæsthesia over the erythematous patch nor over any other part of the body, nor is there any thickening of the nerves. A careful examination of the scrapings from the face showed the presence of a few Leishman-Donovan bodies after a prolonged examination. No lepra bacilli were found from these scrapings.

The case is of much interest as such cases rarely come under observation. Generally speaking, cases that present themselves for treatment show definite de-pigmented areas, with or without nodules either inside them or in independent foci in the skin.

[*Reprinted from Transactions of the Royal Society of Tropical Medicine and Hygiene, Vol. XXIII, No. 3, November, 1929*]

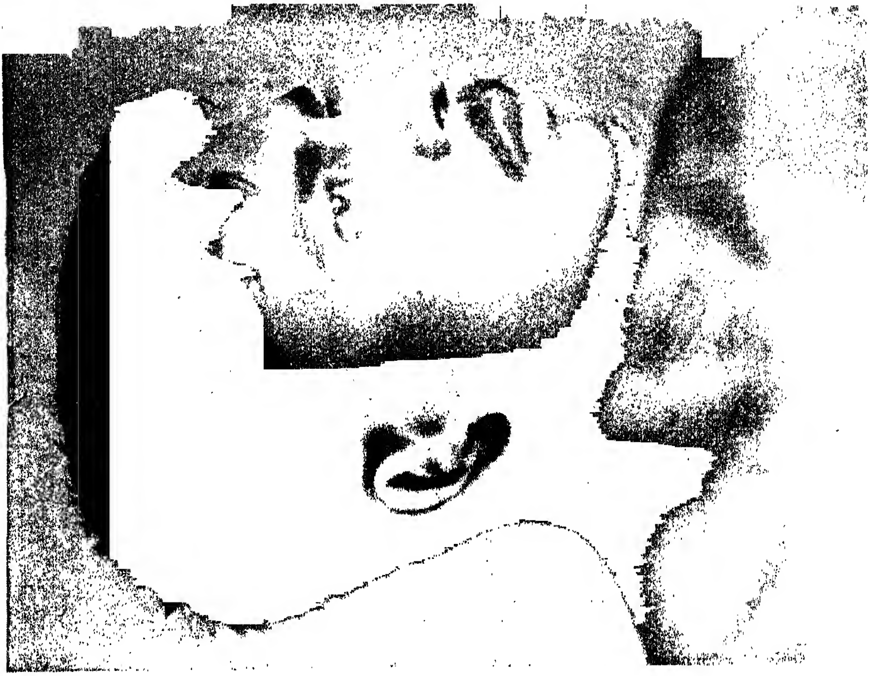
# STUDIES IN KALA-AZAR AND CHEMOTHERAPY OF ANTIMONY

## I. SUBSEQUENT HISTORY OF THE FIRST RECORDED CASE OF DERMAL LEISHMANOID

## II. SUBSEQUENT HISTORY OF A CASE OF DERMAL LEISHMANOID ORIGINALLY CONSIDERED TO HAVE BEEN REFRACTORY TO TREATMENT

### I. Subsequent History of the First Recorded Case of Dermal Leishmanoid

The first recorded case of dermal leishmanoid was published by Brahmachari in the *Indian Medical Gazette* in April, 1922, and described in greater detail in the *Indian Journal of Medical Research* in April, 1923. The case remained under treatment for irregular periods, and was afterwards lost sight of. The following subsequent history has been obtained from one of his relations. The de-pigmented patches increased in size in course of time, and in some places gave rise to typical leucoderma-like areas. The nodules ulcerated, giving rise to sanious foul-smelling discharge. They subsequently diminished in size, and the ulcers healed up. The patient became more and more wasted, and died last February of an attack of dysentery. This is perhaps the first case of dermal leishmanoid in which ulceration ultimately took place in the affected areas of the skin after a long period. As, however, the case was not seen by us in the stage of ulceration, the



authenticity of the formation of ulcers must remain a doubtful point.

## II. Subsequent History of a Case of Dermal Leishmanoid originally considered to have been Refractory to Treatment

This case came under the observation of Brahmachari in July, 1926. Patient was originally treated in the Calcutta School of Tropical Medicine, and described by Acton and Napier in the *Indian Journal of Medical Research* in July, 1927 (Plate). The picture of the patient was subsequently reproduced from this Journal in *Manson's Tropical Diseases*, Ninth edition, 1929. The patient was then considered to have resisted all forms of treatment. The treatments adopted by the above observers are not stated.

*The First Stage of Observation*—

This was the stage at which the patient came under the observation of Brahmachari for the first time up to the commencement of treatment with urea stibamine—the stage of kala-azar with dermal lesions due to *Leishmania-donovani*.

Patient was suffering from kala-azar with fever and enlargement of spleen. The peripheral blood gave positive flagellate culture. Patient had very well-marked nodular eruptions over his face, the trunk and the extremities. Besides these, there were patches of de-pigmentation over the body, especially the trunk.

Blood examination on 28th July, 1928, showed:—Red blood corpuscles—4,000,000; white blood corpuscles—2,700; haemoglobin—75 per cent. Leucocyte count percentages were: Polymorphonuclears—61; mononuclears—18; lymphocytes—17; eosinophiles—4. No malarial parasites.

Wassermann reaction—negative. The nodules in the skin showed *Leishmania-donovani* in smears and flagellates in culture.

Patient was put on a course of treatment with urea stibamine from 15th August, 1926.

*The Second Stage of Observation*—

This was the stage during which the patient underwent treatment with urea stibamine and passed toward its end into the stage of dermal leishmanoid with complete sterilization of the internal organs against leishmania.

At the beginning of this stage the face and the trunk showed nodular eruptions and patches of de-pigmentation. The eruptions were very similar to those in the first stage. During treatment with urea stibamine the spleen quickly diminished in size, and the fever stopped. The skin eruptions seemed to be resistant to treatment, though they slowly and slightly diminished from the trunk and the extremities. Blood examination on 13th September, 1926, after administration of 1·1g. of urea stibamine showed the follwing :—Red blood corpuscles—4,500,000 ; white blood corpuscles—6,600 ; haemoglobin—75 per cent. Leucocyte count percentages were :—Polymorphonuclears—61 ; mononuclears—18 ; lymphocytes —19 ; eosinophiles—2.

Blood culture for flagellates—negative. No fever, no enlargement of the spleen at the end of this stage.

At the end of this stage the case was completely cured of kala-azar, but was still suffering from dermal leishmanoid.

*The Third Stage of Observation*—

This was the stage of dermal leishmanoid during which the patient underwent further treatment with urea stibamine and passed toward its end into the fourth stage, when he was completely cured of dermal leishmanoid.

We persisted in treatment with urea stibamine and after the further administration of 9·3 g. of urea stibamine extending over a period from 13th September, 1926, to 29th

August, 1927, the patient was completely cured. At the end of this stage there was complete sterilization of the tissues against leishmania.

*The Fourth Stage of Observation*—

This was the stage of complete cure and sterilization of all the tissues against leishmania.

The patient was examined by Brahmachari on 1st May, 1929, about eight months after completion of treatment. He was then a perfectly healthy man, without any enlargement of the spleen and with complete disappearance of all traces of dermal leishmanoid (Plate II).

Plate I represents the appearances of the skin eruptions over the face of the patient as reproduced in Manson's *Tropical Diseases*, 1929, from Acton and Napier's paper in the *Indian Journal of Medical Research*, July, 1927. It is reproduced here for purposes of comparison.

Blood examination on 1st August, 1927 was as follows :—Red blood corpuscles—5,000,000; white blood corpuseles—7,500; haemoglobin—100 per cent. Leucocyte count percentages were :—Polymorphonuclears—60·0; large mononuclears—3·2; lymphocytes—33·6; eosinophiles—3·2.

Culture of the peripheral blood—negative.

The case is of great interest as being one in which well-marked nodules containing Leishman-Donovan bodies were observed in the skin at a stage when the patient was not cured of internal leishmaniasis. The skin eruptions still persistad after the patient had been cured of kala-azar and the internal organs had been sterilized against leishmania, the case passing into the condition of dermal leishmanoid. The subsequent history of the case is also of much interest. Originally considered to have been absolutely refractory, the case was completely cured of dermal leishmanoid after a prolonged course of treatment with urea stibamine. Eight

months after the completion of treatment the patient showed no sign whatever of leishmania infection.

*N.B.*—A word about the nomenclature of the disease. Various names have been suggested, one of the latest being " post-kala-azar dermal leishmaniasis " (Acton and Napier, 1927). Very recently, in discussing the various names suggested, the Editor, *Indian Medical Gazette* (1928), pointed out that the name " post-kala-azar dermal leishmaniasis was a clumsy name, though it defined the condition; the alternative, post-generalised dermal leishmaniasis was certainly worse." The name " *leishmanide* " suggested by him is not free from difficulties for the following reasons : A name ending in *ide* frequently gives an impression of an *amide*, such as tryparsamide, and confusion may arise in the mind of the reader as to whether *leishmanide* is the name of a specific for leishmaniasis or the name of a cutaneous manifestation of infection with leishmania. We are therefore inclined to think that on the whole " dermal leishmanoid " is the most appropriate, if not the most convenient, name for the disease. At any rate it has now the sanction of usage, and most observers recognise the disease under that name.

[*N. B.*—This case is the continuation of the one already described in pp. 207-209 of this work.—*Ed.*]

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EDITORIAL RECORD, *Indian Journal of Medicine*, September, 1926.

[*Reprinted from Transactions of the Royal Society of Tropical Medicine and Hygiene, Vol. XXIII, No. 6, April, 1930*]

# STUDIES IN KALA-AZAR AND CHEMOTHERAPY OF ANTIMONY

## PART II

## THE TREATMENT OF KALA-AZAR WITH INTRAMUSCULAR INJECTION OF SODIUM-N-PHENYL-GLYCINE-AMIDE-4-STIBINATE

In a paper published in the *Indian Journal of Medicine*, June, 1926, and in the *Calcutta Medical Journal*, June, 1926, Brahmachari and his assistants described a series of eight cases of kala-azar successfully treated with intravenous injection of *sodium-N-phenyl-glycine-amide-4-stibinate* (the antimony analogue of tryparsamide).

### INTRAVENOUS INJECTIONS

The notes of these published cases are summarized below for reference.

*Case I.*—D., æt. 16, was admitted, with history of fever for six months. The spleen extended 5 in. below the costal arch. Blood culture on NNN medium gave a positive flagellate culture. Patient was treated with intravenous injections of which twenty were given. The fever completely stopped, the spleen ceased to be felt below the costal arch, and at the time of discharge blood culture on NNN medium was negative. Increase of weight during treatment—1 st. 1 lb. Dose—0·05 to 0·2 gram.

*Result of Blood Examination*—

(1) R.B.C.—1,800,000, W.B.C.—2,500, Hb.—35 per cent, on 11th February, 1925, before treatment.

(2) R.B.C.—4,500,000, W.B.C.—8,400, Hb.—85 per cent, on 20th June, 1925, after treatment.

*Case II.*—J., æt. 10, was admitted on 17th November, 1924, with history of fever for five months. The spleen extended 4½ in. below the costal arch. Patient was treated with fifteen intravenous injections. The fever stopped after the third injection. Increase in weight during treatment—1 stone. Dose—0·05 to 0·2 gram.

*Result of Blood Examination*—

(1) R.B.C.—2,500,000, W.B.C.—3,500, Hb.—45 per cent, on 19th November, 1924, before treatment.

(2) R.B.C.—4,400,000, W.B.C.—9,600, Hb.—45 per cent, on 27th March, 1925, after treatment.

*Case III.*—S., æt. 14, was admitted on 3rd May, 1925, with history of fever for three months. The spleen extended 2½ in., and the liver 1 in. below the costal arch. Patient was treated with twelve intravenous injections. The fever stopped after the fourth injection. Dose—0·05 to 0·20 gram.

*Result of Blood Examination*—

(1) R.B.C.—2,000,000, W.B.C.—1,800, Hb.—40 per cent, on 20th June, 1925, before treatment.

(2) R.B.C.—4,200,000, W.B.C.—7,100, Hb.—70 per cent, on 12th August, 1925, after treatment.

*Case IV.*—J., æt. 10, was admitted on 2nd March, 1925, with history of fever for four months. The spleen extended to the umbilicus, and the liver was just palpable below the costal arch. He was treated with ten intravenous injections. The temperature came down to normal after the

fourth injecton. At the time of discharge, the spleen and the liver could not be felt below the costal arch. Dose—0·05 to 0·2 gram.

*Result of Blood Examination*—

(1) R.B.C.—2,500,000, W.B.C.—2,500, Hb.—45 per cent, on 8th March, 1925, before treatment.

(2) R.B.C.—4,000,000, W.B.C.—5,100, Hb.—75 per cent, on 10th May, 1925, after treatment.

*Case V.*—M., æt. 20, was admitted on 25th May, 1925, with history of fever for two years. The spleen extended 6 in. below the costal arch. Patient was treated with fifteen intravenous injections. The temperature came down to normal after the fifth injection. At the time of discharge the spleen was felt 2 in. below the costal arch. Dose—0·05 to 0·20 gram.

*Result of Blood Examination*—

(1) R.B.C.—2,500,000, W.B.C.—2,100, Hb.—45 per cent, on 27th May, 1925, before treatment.

(2) R.B.C.—3,200,000, W.B.C.—6,500, Hb.—70 per cent, on 17th August, 1925, after treatment.

*Case VI.*—S., æt. 21, was admitted on 28th February, 1925. Both spleen and liver extended 4 in. below the costal arch. He was treated with fourteen intravenous injections. The temperature came down to normal after the fifth injection. At the time of discharge the spleen was felt 1 inch below the costal arch. Dose—0·05 to 0·2 gram.

*Result of Blood Examination*—

(1) R.B.C.—2,820,000, W.B.C.—1,800, Hb.—30 per cent, on 31st March, 1925, before treatment.

(2) R.B.C.—3,000,000, W.B.C.—5,000, Hb.—65 per cent, on 29th May, 1925, after treatment.

*Case VII.*—B., æt. 12, was admitted on 23rd March, 1925. The spleen extended 8 in. and his liver 2 in. below the costal arch. Patient was jaundiced. He was treated with ten intravenous injections. The temperature came down to normal after the sixth injection. At the time of discharge the spleen could not be felt below the costal arch. Increase of weight after treatment—1 st. 7lbs. Dose—0·05 to 0·2 gram.

*Result of Blood Examination*—

(1) R.B.C.—2,500,000, W.B.C.—2,800, Hb.—20 per cent, on 26th March, 1925, before treatment.

(2) R.B.C.—4,500,000, W.B.C.—6,200, Hb.—80 per cent, on 10th June, 1925, after treatment.

## Intramuscular Injections

The above cases showed satisfactory results obtained by intravenous injection in the treatment of kala-azar. As the compound is the antimony analogue of tryparsamide, the possibility of using it *intramuscularly* with advantage was suggested by Brahmachari. There was for some time considerable difficulty in preparing the compound in a pure state. This was subsequently overcome, and the chances of local irritation after intramuscular injection by the presence of minute impurities eliminated as much as possible.

The following are the notes of five cases of kala-azar successfully treated by intramuscular injections. The first case was communicated by Brahmachari to the International Congress of Tropical Medicine and Hygiene, held at Cairo, in December, 1928 and is quoted here for reference.

*Case I.*—Patient, æt. 25, came under the treatment of Brahmachari, with history of fever lasting for about six months. The spleen extended 5 in. and the lever 3 in. below

the costal margin and Leishman-Donovan bodies were found on spleen puncture. Blood picture at the commencement of treatment was : R.B.C.—3,500,000 ; W.B.C.—3,500 ; Hb.—45 per cent. Patient was given injections in doses of 0·1 to 0·3 gram subcutaneously and intramuscularly. Temperature came down to normal after the fourth injection. After the twelfth injection, the spleen could not be felt below the costal margin, and no Leishman-Donovan bodies could be found on spleen puncture. The blood at the time of communication to the Congress (three weeks after the commencement of treatment) showed : R.B.C.—3,500,000; W.B.C.—6,500; Hb.—65 per cent. Generally speaking, there was very little local reaction at the seats of subcutaneous or intramuscular injection. The patient was completely cured at the time of writing the present paper.

*Case II.*—H. C. M. came under the treatment of Brahmachari on 3rd January, 1929, with history of fever of four months' duration. Spleen at the time of first observation extended 4 in. and liver 2 in. below the costal margin. Result of blood examination : R.B.C.—315,000 ; W.B.C.—3,200; Hb.—45 per cent, on 10th January, 1929. Spleen puncture—Leishman-Donovan bodies present. Patient was put on treatment with fifteen intramuscular injections in doses of 0·05 to 0·15 gram twice a week. The temperature came down to normal after the fourth injection. On 7th April, 1929, at the time of discharge, spleen and liver could not be felt below the costal margin. Blood examination :—R.B.C.—4,500,000 ; W.B.C.—8,000 ; Hb.—75 per cent. Peripheral blood culture—negative for flagellates.

The Notes of three cases recently treated in the Chittaranjan Hospital, Calcutta, are given below :—

*Case III.*—R.C.J., æt. 10, was transferred from the Cholera Ward to the Kala-azar Ward of the Chittaranjan Hospital on 24th June, 1929. His leucocyte count at the

beginning of the treatment was 3,100. The spleen was 4 in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given altogether eleven injections in doses of 0·05 to 0·1 gram. Temperature came down to normal after the second injection and it continued to be so except for one sharp rise which occurred after the fifth injection; but it rapidly went down to normal. At the time of discharge the leucocyte count was 7,200, and the spleen could not be felt below the costal arch. No Leishman-Donovan bodies could be found on spleen puncture. Increase in weight—1 stone.

*Case IV.*—K. D., æt. 21, was admitted into the Kala-azar Ward of the Chittaranjan Hospital, with a history of irregular attacks of fever for six months. His spleen was felt 6 in. and the liver 2 in. below the costal margin. Many Leishman-Donovan bodies were found on spleen pucture. Leucocyte count at the beginning of treatment was 3,100. Patient was given thirteen intramuscular injections in doses of 0·1 to 0·15 gram. At the end of treatment spleen and liver could not be felt below the costal margin. Result of blood examination :—R.B.C.—4,600,000; W.B.C.—6,500; Hb.—90 per cent. Spleen puncture—negative. Increase in weight—2 stone.

*Case V.*—B.B.S., æt. 18, was admitted on 19th November, 1929, into the Kala-azar Ward of the Chittaranjan Hospital, with history of fever for one month and with spleen enlarged to about 3½ in. and liver 2 in. below the costal margin Spleen puncture showed a large number of Leishman-Donovan bodies. Blood examination at the beginning of treatment :—Leucocyte count—4,500; Differential count : polymorphonuclears—50 per cent; lymphocytes —44 per cent; mononuclears—6 per cent.; poikilocytes, anisocytes, normoblasts, myelocytes and basophiles present. Patient was put on intramuscular injections in doses of 0·05 to 0·1 gram. Temperature came down to normal after the

fifth injection. Patient was given altogether fifteen injections in doses of 0·05 to 0·1 g. At the time of writing, spleen and liver could not be felt below the costal margin. Result of blood examination : R.B.C.—4,500,000; W.B.C.—7,500 ; Hb.—80 per cent. Spleen puncture—negative.

## OBSERVATIONS

Sodium-N-phenyl-glycine-amide-4-stibinate. The antimony analogue of tryparsamide has been successfully used by the *intramuscular method* in the treatment of kala-azar. The local irritation caused by it is frequently slight. The same drug has also been successfully used by the intravenous method in the treatment of kala-azar.

The maximum tolerated dose in the case of the white rat given intravenously is 0·3 gram per kg. of body weight.

Many antimony preparations have been used from time to time intramuscularly for the treatment of kala-azar. A Reference to their use is given below :—

1. Castellani's solution of tartar emetic.
2. Brahmachari's hyperacid antimonyl tartrate.
3. Sodium antimonyl tartrate.
4. Acetyl-*p*-amino-phenyl-stibinate of sodium (Stibenyl).
5. Urea stibamine.
6. Neo-stibosan.

Many of the above compounds have been found to give rise to much local irritation, and up to now none has yet been popular for the purpose of intramuscular injection.

In view of the fact that the present compound is allied to tryparsamide, its value in the treatment of kala-azar by the intramuscular route cannot be over-estimated. Its use is indicated in individuals with thin veins or in whom intra-

venous injections of any antimony compound are followed by severe constitutional symptoms. The compound was first discovered by Brahmachari in 1922.

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# STUDIES IN KALA-AZAR AND CHEMO-THERAPY OF ANTIMONY

## PART III

### OBSERVATIONS ON ANTIMONY IN THE SPLEEN CELLS OF ANIMALS INFECTED WITH *LEISHMANIA-DONOVANI*

The pathology of the spleen in kala-azar is an important subject for research. In experimentally infected animals the spleen may show little or no fibrous tissue development, but contains in heavily infected cases, a very large number of cells harbouring *Leishmania-Donovani*. These cells possess the property of picking up particles of antimony when it is introduced into the circulation in a state of fine subdivision, as will be seen from the experiments described below.

Metallic antimony in a state of fine subdivision was prepared according to the method of Plimmer (1911) in the following way:

"By dissolving antimony trichloride or other salts of antimony in hydrochloric acid or other acids diluted with water or an aqueous solution of an organic acid such as tartaric acid and adding zinc to the solution, the antimony is precipitated in a finely divided state. The proportions employed are 50 grams of antimony trichloride, 100 c.cm. of concentrated hydrochloric acid, diluted with 200 c.cm. of water or 200 c.cm. of 2 to 5 per cent tartaric acid solution and 25 to 30 grams of zinc. When the zinc is completely dissolved, the antimony is filtered off and

washed with an aqueous solution of an organic acid, such as tartaric acid, until free from chlorides, and then with water till free from acid. The antimony thus obtained is pure and in a finely divided state."

One per cent suspension of metallic antimony prepared in the above way was made in normal saline in a test-tube and the heavier particles were allowed to settle for ten to fifteen minutes at the bottom of the tube.

The supernatant suspension was injected intravenously into healthy as well as leishmania-infected mice in doses of 20 c.cm. per kilo. of body weight.

The animals were killed with coal gas at intervals of two hours, two days and seven days after injection, and sections of the organs were then prepared.

No antimony was found inside the cells of the spleen two hours after injection. On the other hand, two days and seven days after injection antimony was found inside certain cells of the lungs and spleen.

The section of spleen shown here (see Plate) is that of a leishmania-infected mouse killed forty-eight hours after intravenous injection of metallic antimony in doses of 20 c.cm. per kilo. of body weight on two successive days, and stained with iron-hæmatoxylin. On careful examination the following types of cells were found inside the spleen of the mouse. (Some of the cells are shown in the Plate.)

1. Large cells with faintly stained cytoplasm containing leishmania as well as antimony particles.

2. Cells containing antimony in a diffuse (probably colloidal) state, which may or may not harbour leishmania.

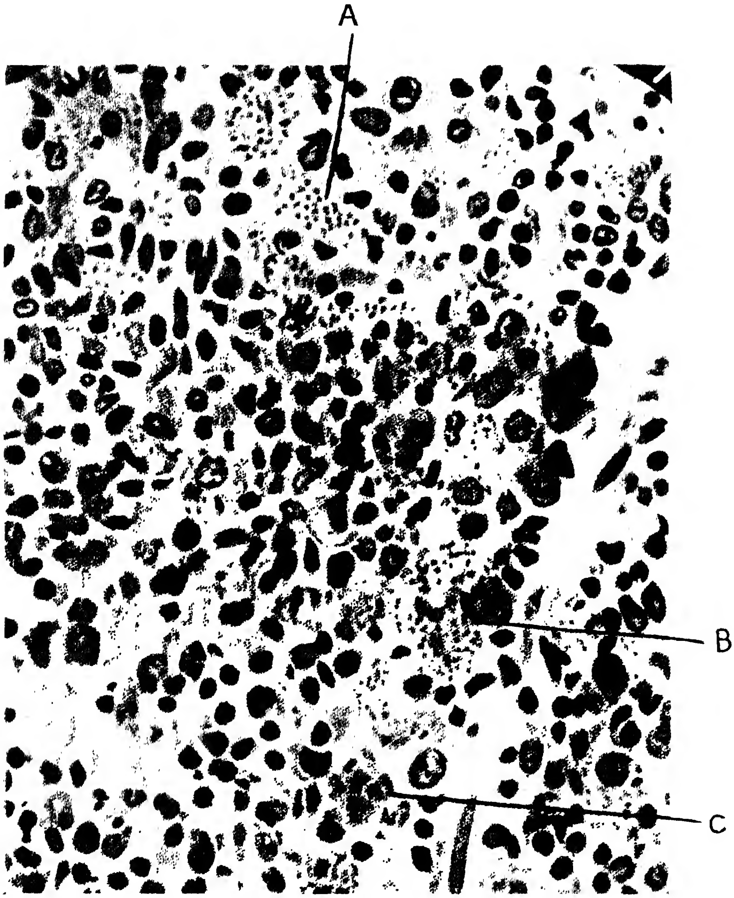
3. Cells of moderate size containing a large number of leishmania as well as particles of antimony.

4. Certain small cells containing particles of antimony, although they do not contain any leishmania.

5. Large cells with faintly stained cytoplasm containing very few or no antimony particles, but full of leishmania.

[*Reprinted from the Proceedings of the Twenty-fifth Indian Science Congress, 1933, Part II, pp 258-302*]

# Plate




Photomicrograph of spleen of a mouse infected with *Leishmania Donovani* forty-eight hours after intravenous injection of metallic antimony.

A. Cell containing leishmania, but no particles of antimony.

B. Cell with faintly stained cytoplasm containing leishmania and a few particles of antimony.

C. Cell containing coarse granules of antimony and leishmania, some of which appear to be degenerated.

(Reproduced from a paper by the author and co-workers published in the *Transaction of the Royal Society of Tropical Medicine and Hygiene*, Vol. XXIII, No. 6, April, 1930.)

6. Certain fairly large cells with particles of antimony and degenerate leishmania inside them.

7. Giant cells containing very few or no antimony particles or leishmania.

8. Cells with fairly large and well-stained nuclei containing neither antimony nor leishmania.

In certain places there were particles of antimony free in blood spaces inside the spleen. We have further observed that cells harbouring the largest number of leishmania do not necessarily pick up the largest number of particles of antimony.

Some of the above cells are probably of the nature of clasmatocytes.

From the above it will be seen that antimony was present inside the cells of the spleen in two forms: (1) as a diffuse brownish-yellow stain and (2) as black granules. We consider that the diffuse staining is due to the metallic antimony being converted into a colloidal form by the cells of the spleen in the process of its internal metabolism before it is converted into a soluble compound.

These observations appear to have an important bearing upon the action of antimony in the treatment of kala-azar.

Levaditi has propounded a general law with reference to all the members of the nitrogen family of elements occupying Group V of Mendeleff's periodic table—arsenic, antimony, vanadium, bismuth, etc. They or their compounds exhibit paraciticidal properties after they have been acted upon by certain cells. It is probable that some of these cells are clasmatocyte cells giving rise in the case of bismuth to a toxalbumin, the bismoxyl of Levaditi, which possesses destructive power against the *Treponema pallidum*.

It has been suggested by Brahmachari elsewhere that an antimony compound, in order that it may be of therapeutic value, must be converted in the tissues into a

compound containing the radical $-Sb=O$ in the reactive stage. Chemically, some of the therapeutic antimony compounds contain the radical $-Sb=O$, and the therapeutic bismuth compounds the radical $-Bi=O$. It is likely that bismoxyl contains the radical $-Bi=O$ in the reactive stage, and that a corresponding antimony compound which has been called *stiboxyl* (Brahmachari, 1928) is probably formed in the case of antimony. It has been observed that metallic bismuth, finely subdivided, is more suitable for the production of bismoxyl when administered intramuscularly than in the form of a chemical compound. The same could be expected of metallic antimony, but for the fact that when injected intramuscularly it gives rise to so very severe local irritation that it is unsuitable for intramuscular injection for therapeutic purposes. On the other hand, metallic antimony injected intravenously is one of the most powerful antimonials in the treatment of kala-azar, as was shown by Brahmachari (1915) some years ago. It has been observed by Meleney that in kala-azar clasmatocyte tissue is developed as a tissue reaction. Probably this reticulo-endothelial system gives rise to the production of *stiboxyl* in the spleen and elsewhere. If this view be correct, then it may be concluded that individual cases will get beneficial results from the use of antimony compounds proportional to the reaction of the reticulo-endothelial system. The following two requirements are therefore necessary for an antimonial to be effective, namely: (1) the development of the clasmatocytes, and (2) the introduction of an antimony compound with which they can combine for the development of *stiboxyl* and the ability of the clasmatocytes to metabolise the antimonial and give rise to one which will act upon the parasites. Herein lies the value of the different antimonials in the treatment of kala-azar. This will explain why with the same antimony compound, one individual is cured much more quickly than another after its administration. It is the

development of these tissue cells and their ability to metabolise the compound used that should be aimed at in the treatment of the disease. It is clear from the above observations that after intravenous administration of metallic antimony into an animal experimentally infected with kala-azar, the leishmania come into closest contact with the particles of antimony inside certain tissue cells, and are subsequently destroyed by a highly reactive antimony compound formed inside them in the process of metabolism inside these cells. How these cells may be developed and how their ability to metabolise antimony compound picked up by them may be increased is a subject for further research.

## Observations

Metallic antimony injected intravenously in a state of fine subdivision into leishmania-infected mice is picked up inside the spleen by cells that harbour leishmania and an antimony compound is developed inside the cells during the process of metabolism which kill the leishmania. In certain cells inside the spleen metallic antimony is found in a diffuse state, and this is probably the stage in which the solid finely divided antimony is converted into colloidal particles before passing into complete solution. Cells harbouring the largest number of leishmania do not necessarily pick up the largest number of particles of antimony. Degenerate leishmania have been observed inside cells containing particles of antimony.

The senior author's grateful thanks are due to Major Shortt, Director, Kala-azar Commission, Assam, for providing him with leishmania-infected mice. Without his aid, it would not have been possible to conduct the above work. He is also grateful to Dr. Baini Prashad of the Zoological Survey of India, for the beautiful photo-micrograph of the section of

spleen of the infected mouse which is attached to the present paper.

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# STUDIES IN KALA-AZAR AND CHEMO-THERAPY OF ANTIMONY

## PART IV

### FURTHER OBSERVATIONS ON THE ANTIMONY-LADEN CELLS OF SPLEEN AFTER INTRAVENOUS INJECTION OF METALLIC ANTIMONY IN A STATE OF FINE SUSPENSION IN EXPERIMENTAL ANIMALS

In our last paper communicated to these Transactions (Vol. XXIII, No. 5) we found that when metallic antimony in a fine state of subdivision is injected intravenously into a leishmania-infected mouse, many of the antimony particles are picked up inside the spleen by cells containing leishmania, and others which do not contain them. These cells are probably clasmatocytes.

In the present paper the results of observations on healthy mice after intravenous injections of finely divided metallic antimony are recorded. The same procedure as was adopted in our former paper was followed in our present observations. One per cent suspension of finely divided metallic antimony, prepared according to the method of Plimmer, was made in normal saline solution, and then allowed to settle for ten to fifteen minutes, and the supernatant suspension was injected into the dorsal vein of the tail of a mouse in doses of 20 c.cm. per kilo. The injection was repeated after twenty-four hours, and the animal killed forty-eight hours after the first injection.

In sections of the spleen of these experimental animals we find that the particles of antimony are picked up by cells

similar to those in the case of leishmania-infected mice, with the difference that in the case of healthy mice a larger number of particles of antimony are extracellular forty-eight hours after the first and twenty-four hours after the second injection than in the case of infected mice. This is explained by the fact that in the infected mice, a larger number of cells of the nature of clasmatocytes, which are capable of taking up leishmania as well as antimony particles, are developed in them as a result of tissue reaction than those present in healthy mice. Further, it has been observed that in the case of infected mice these cells are much larger in size, and the particles of antimony inside them are more dispersed than in the case of healthy mice, in which they are present in a more compact state. (Compare the Photomicrograph in our previous paper on the subject with that in the present paper.) This may be explained by assuming that the leishmania-laden clasmatocytes differ in the degree of their functional activity from those present in healthy mice.

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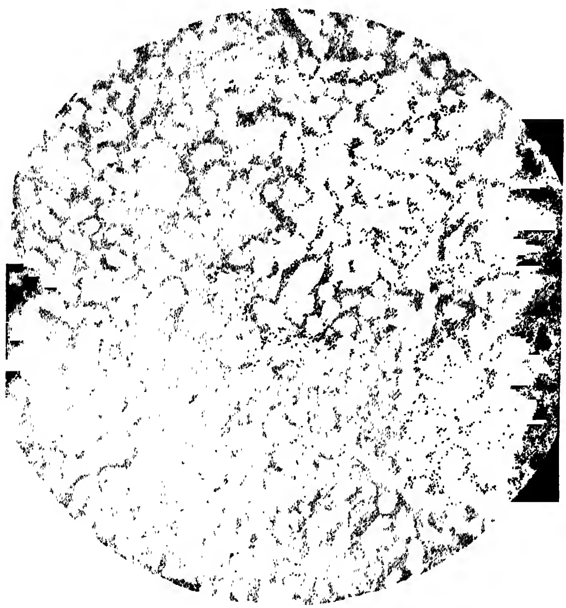

Photomicrograph of spleen of a mouse infected witb Leishmania Donovani, forty-eight hours after intravenous injection of metallic antimony

[*Reprinted from Transactions of the Royal Society of Tropical Medicine and Hygien Vol. XXIV, No. 5, March, 1931, and the Indian Journal of Medicine, Vol. XII, June, 1931*]

# STUDIES IN KALA-AZAR AND CHEMO-THERAPY OF ANTIMONY

## PART V

### THE TREATMENT OF RESISTANT CASES OF DERMAL LEISHMANOID*

In treating cases of kala-azar with antimonial preparations, the authors have met with the following types: (1) Those which quickly yield to antimonial treatment; (2) those which resist for a considerable period and slowly yield to treatment; (3) those which seem to be extremely or absolutely resistant; (4) those which relapse after insufficient or improper treatment and are either very resistant to subsequent treatment or quickly yield to it. Thus patients vary in their response to treatment, while the possibility of antimony-resistant leishmania, which may be present from the very beginning or develop during treatment, has to be considered. Relapses occur after too early abandonment of treatment, and also, but rarely, in cases which, from the marked improvement in the general condition and in the blood picture and freedom from fever for some months, appear to have been cured.

As regards dermal leishmanoid, contrary to the opinion of Muir (1930), it is found that most cases take much

* Read at tha Medical Section of the Indian Science Congress held at Nagpur, January, 1931.

longer to cure than cases of kala-azar. This fact is significant, for the disease is more common than was supposed when it was first discovered (1922). The unsightly nature of the lesions makes the patients very unhappy as well as objectionable to others. Furthermore, such cases are probable sources of infection, so that the need for a rapid cure cannot be over-emphasized. A most resistant case of dermal leishmanoid which was originally considered by Acton and Napier as absolutely refractory to treatment, has been previously recorded in this Journal (1929). The course of treatment adopted was not indicated by these authors. In a more recent communication, however, reference is made to the case by Napier and Haldar (1930). It is stated that between fifty and sixty injections of different antimony compounds were given. Urea stibamine alone is named, and of this the patient is stated to have had twelve injections. As noted (1929), this case was subsequently cured by a very prolonged course of urea stibamine. It is evidently important to discover methods for reducing the course of treatment.

This paper refers to those cases of dermal leishmanoid which show little or no improvement after continuous antimonial treatment extending over a period of three months or more. The type of case recently mentioned by Napier as having been cured after the usual course for the treatment of kala-azar is not dealt with here, though such has also been observed by us.

On the assumption that skin lesions would respond most quickly if antimony were introduced through the skin, Brahmachari conceived the idea of combining ointment of metallic antimony with intravenous injection of urea stibamine. Six cases have now been treated in this way, and the results appear so far to be much more satisfactory than treatment by the intravenous route alone. The notes of one of the most resistant of these cases are given here.

The patient came under observation in January, 1929, with well-marked dermal lesions due to *Leishmania-donovani*. He gave a history of having had kala-azar about two years previously, and of being cured after antimonial treatment. After forty injections of urea stibamine in doses of 0·1 to 0·82 gram he was much improved. Treatment was discontinued for six months, after which there were well-marked nodules on his face and marked patches of de-pigmentation all over the body. The patient then received during two months three injections of urea stibamine and twelve of neo-stibosan, but as improvement was very slight, a combined treatment of metallic antimony inunction (5 per cent) and intravenous injection of urea stibamine was instituted. After twenty-seven injections had been given all the nodules on the skin had disappeared. The patient is still under our observation.

In addition to the above treatment, we have in a few cases tried berberine sulphate—a drug used by old writers for the treatment of enlarged spleen in India. O'Shaughnessy recommended it for the treatment of ague and remittent fevers, while in the early days of the antimony treatment of kala-azar, Brahmachari used this drug intravenously in the treatment of kala-azar without any benefit (1916). Berberine has, however, had a long-standing reputation for being useful in the treatment of oriental sore, and is obtainable in the market in a crude state under the popular name of *rasaut*. Recently acid berberine sulphate has been used with success in the treatment of the same disease, both as an ointment and by infiltration of the sore. It is now obtainable under the name of *orisol*. We have used this in a few cases of dermal leishmanoid for the treatment of the de-pigmented patches, but up to the present no benefit has been obtained from the use of this drug intravenously or subcutaneously.

Metallic antimony used by us was in the form of an

impalpable powder prepared by the method of Plimmer, which has already been described by us in this Journal (1930). The ointment used contains 5 per cent of metallic antimony in equal parts of lanoline and vaseline. It is gently rubbed over the affected parts of the skin for ten to fifteen minutes daily till the skin lesions disappear. In two cases, we injected 0·125 to 0·25 c.cm. of a 5 per cent solution of urea stibamine into some of the nodules, and this was followed in a few days by marked shrinkage without any local irritation.

## Conclusions

In order to shorten treatment, cases of dermal leishmanoid should be treated by metallic antimony inunction combined with intravenous injection of a therapeutic aromatic pentavalent antimonial, which, in our cases, consisted mostly of urea stibamine.

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# STUDIES IN KALA-AZAR AND CHEMO-THERAPY OF ANTIMONY

## PART VI

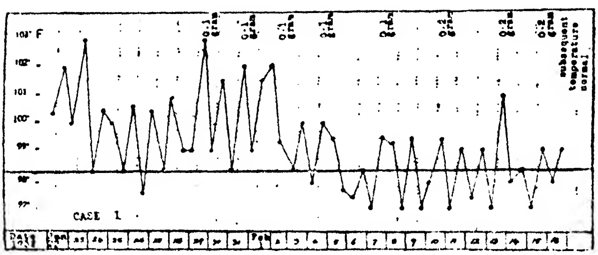
### TREATMENT OF KALA-AZAR WITH INTRAMUSCULAR INJECTION OF SODIUM SULPHOMETHYL STIBANILATE

In these Transactions, Brahmachari and his co-workers described in 1930 a series of cases of kala-azar successfully treated with intramuscular injection of sodium-N-phenyl-glycine-amide-4-stibinate, the antimony analogue of tryparsamide. Excepting this arsenic compound, the organic therapeutic arsenicals are generally derivatives of arsenobenzene. On the other hand the organic therapeutic antimonials are generally derivatives of *p*-stibanilic acid. Among the therapeutic aromatic arsenicals, sodium-methylene-sulphonic acid derivative of dioxy-diamino-arsenobenzene or di-sodium-dioxy-diamino-arsenobenzene-methylene-sulphonate has been successfully used intramuscularly in the treatment of syphilis under the various trade names of thio-sarmine, sulfarsenol, etc., and it occurred to the writer to give a trial to sodium-methylene-sulphonic acid derivative of stibanilic acid or sodium-sulphomethyl-stibanilate in the treatment of kala-azar, As stibinobenzene compounds are unstable, they have not yet come into use in therapeutics and no attempt was made to synthetize them.

The synthesis of sodium-sulphomethyl-stibanilate has been successfully effected in the Department of Chemistry of the Brahmachari Research Institute, Calcutta. It is a greyish white heavy powder, freely soluble in water and its solution is very faintly acidic to litmus. Its antimony content is 24·5 per cent.

The following is a series of cases of kala-azar successfully treated with intramuscular injection of this compound.

*Case I.*—A.K. M., male, æt. 12, was admitted into Brahmachari's ward in the Carmichael Medical College Hospitals with history of fever for six months. The spleen was hard and extended 5 in. and liver extended $1\frac{1}{2}$ in. below the costal arch. Leishman-Donovan bodies were found on spleen puncture. Patient was given 21 injections of the compound intramuscularly in doses of 0·1 to 0·3 gramme, at first every other day and subsequently twice a week. Effect of treatment on temperature is shown in the accompanying *Chart*. At the time of discharge, spleen and liver could just be felt below the costal arch.

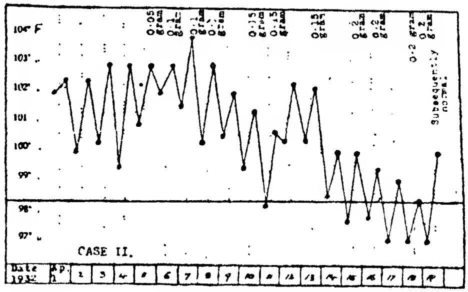



*Total dose*—4·6 grammes.

*Result of Blood Examination*—

Before treatment: R.B.C.—3,375,000; W.B.C.—2,500; Hb.—45 per cent.

After treatment: R.B.C.—4,100,000; W.B.C.—6,380; Hb.—80 per cent.



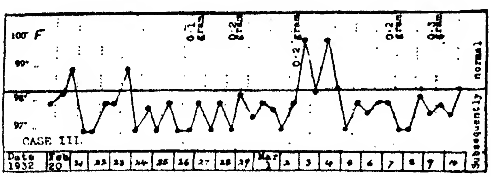

*Case II.*—A. C. M., male, æt. 12, was admitted into Brahmachari's ward in the Carmichael Medical College Hospitals, with history of fever for eight months. The spleen was hard and extended 2 in. and liver also extended 2 in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given fifteen injections of the compound intramuscularly in doses of 0·05 to 0·2 gramme generally every day and sometimes every other day. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could not be felt below the costal arch.

*Total dose*—2·11 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—2,240,000 ; W.B.C.—2,300 ; Hb.—45 per cent.

After treatment : R.B.C.—3,500,000 ; W.B.C.—6,250 ; Hb.—60 per cent.




*Case III.*—S., female, æt. 26, was admitted into the ward of Dr. P. Nandi, Physician, Carmichael Medical College Hospitals, with history of fever for one year and by his kind courtesy the patient was put under my treatment. The spleen was hard and

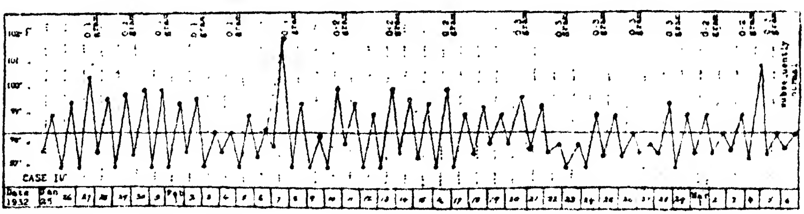
extended 4½ in. and liver extended 1½ in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given thirteen injections of the compound intramuscularly in doses of 0·1 to 0·3 gramme generally every other day. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could just be felt below the costal arch.

*Total dose*—3·7 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—2,290,000 ; W.B.C.—3,450.
After treatment : R.B.C.—4,000,000 ; W.B.C.—5,624 ; Hb.—55 per cent.

*Case IV*.—M.B., male, æt. 30, was admitted into the Carmichael Medical College Hospitals, with history of fever for about a year. There was bleeding from the gums and patient had frequent attacks of severe epistaxis. There was presence of copious albumin in the urine. There was œdema in the extremities. Spleen was hard and extended




4 in. and liver extended 3 in. below the costal margin. Leishman-Donovon bodies were found on spleen puncture. Patient was given seventeen injections of the compound intramuscularly in doses of 0·1 to 0·3 gramme generally every other day. Effect of treatment on temperature is shown in the accompanying Chart. At the time

of discharge, spleen and liver could just be felt below the costal arch, and there was no albumin in the urine, and no Leishman-Donovan bodies were found on spleen puncture.

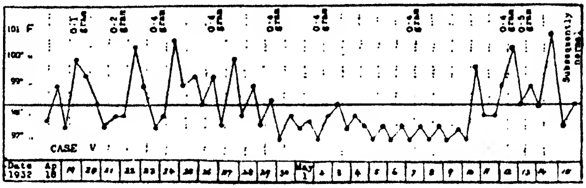
*Total dose*—3·75 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—2,300,000 ; W.B.C.—2,300 ; Hb.— 40 per cent.

After treatment : R.B.C.—3,000,000 ; W.B.C.—5,000 ; Hb.—50 per cent.

*Case V.*—A. C. M., æt. 36, was admitted into the Carmichael Medical College Hospitals, with history of fever for about a year.



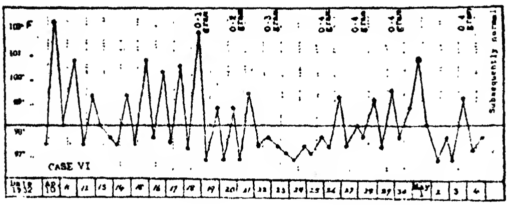

The spleen was hard and extended 2½ in. and liver extended 1 in. below the costal margin. Patient was given nine injections of the compound intramuscularly in doses of 0·1 to 0·5 gramme, at first every other day and subsequently twice a week. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could just be felt below the costal arch.

*Total dose*—3·6 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—3,000,000 ; W.B.C.—2,812 ; Hb —55 per cent.

After treatment : R.B.C.—3,250,000 ; W.B.C.—6,250 ; Hb.—83 per cent,



*Case VI.*—K.D., male, æt. 26, was admitted into the Carmichael Medical College Hospitats, with history of fever for two years. The spleen was hard and extended 5 in. and liver extended 2½ in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture, and in addition there were crescents in the blood. Patient was given eleven injections of the compound intramuscularly in doses of 0·1 to 0·4 gramme, at first every other day and subsequently twice a week, together with a course of treatment with quinine. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could just be felt below the costal arch, and no Leishman-Donovan bodies were found on spleen puncture.

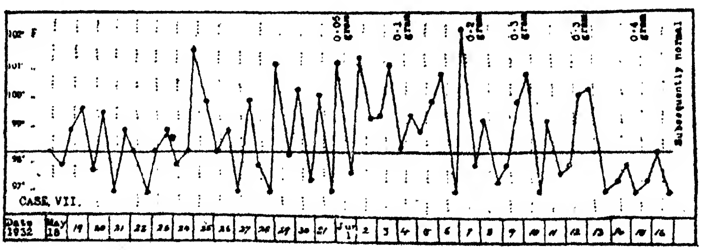
*Total dose*—3·7 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—2,850,000 ; W.B.C.—2,720.

After treatment : R.B.C.—3,250,000 ; WB.C.—6,250 ; Hb.—60 per cent.

*Case VII.*—R.M., male, æt. 20, was admitted into the Carmichael Medical College Hospitals, with history of fever for six months. The spleen was hard and extended



7 in. and liver extended 1 in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given eleven injections of the compound intramuscularly in doses of 0·05 to 0·4 gramme, generally twice a week. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could not be felt below the costal arch.

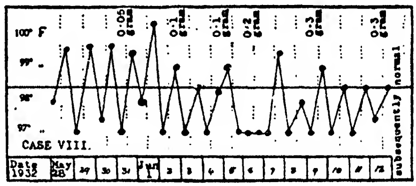
*Total dose*—3·4 grammes.

*Result of Blood Examination*—

Before treatment: R.B.C.—1,450,000; W.B.C.—2,188; Hb.—45 per cent.

After treatment: R.B.C.—3,000,000; W.B.C.—5,000; Hb.—60 per cent.

*Case VIII.*—N., male, æt. 32, was admitted into the Carmichael Medical College Hospitals, with history of fever for six months. The spleen was hard and extended 3½ in. and the liver extended 1½ in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given thirteen injections of the compound intramuscularly in doses of 0·05 to 0·3 gramme, at first every other day and subsequently twice a week. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could not be felt below the costal arch.

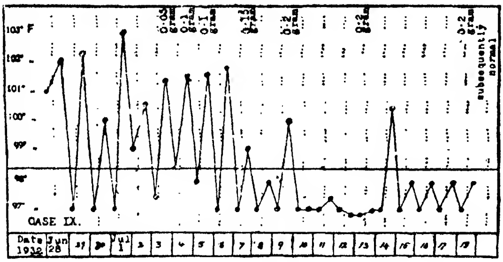



*Total dose*—2·5 grammes.

*Result of Blood Examination*—

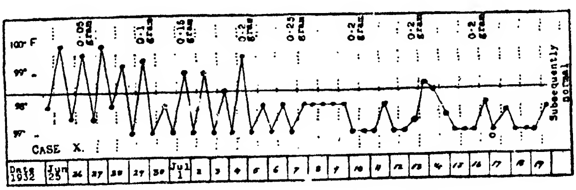
Before treatment: R.B.C.—2,750,000; W.B.C.—2,500; Hb.—50 per cent.

After treatment: R.B.C.—3,100,000; W.B.C.—6,688; Hb.—65 per cent.



*Case IX.*—N.C.P., male, æt. 30, was admitted into the Carmichael Medical College Hospitals, with history of fever for one year. He had attacks of diarrhœa and cough. There was presence of albumin in the urine. The Spleen was soft and tender extending $1\frac{1}{2}$ in. below the costal margin. Liver also extended $1\frac{1}{2}$ in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given seven injections of the compound intramuscularly in doses of 0·05 to 0·2 gramme, at first every day and subsequently every other day and twice a week. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could not be felt below the costal arch and there was no albumin in the urine.

*Total dose*—



*Case X.*—P. P.S., male, æt. 26, was admitted into the surgical wards of the Carmichael Medical College Hospitals for treatment of right perinephritic iliac abscess and subsequently transferred to Brahmachari's ward for treatment of kala-azar. The spleen was hard and extended $3\frac{1}{2}$ in. and liver extended $1\frac{1}{2}$ in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given eight injections of the compound intramus-

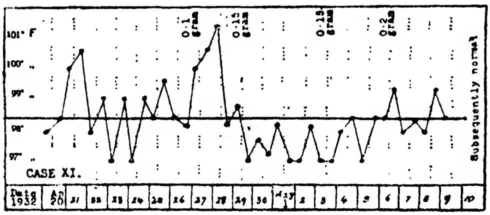
cularly in doses of 0·05 to 0·2 gramme, generally twice a week. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could not be felt below the costal arch.

*Total dose*—1·35 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—2,400,000 ; W.B.C.—3,500 ; Hb.—50 per cent.

After treatment : R.B.C.—3,800,000 ; W.B.C.—8,750 ; Hb.—65 per cent.



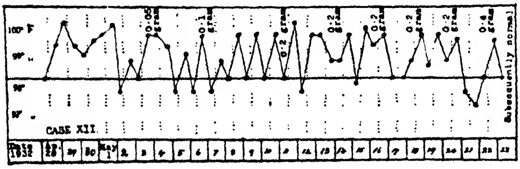

*Case XI.*— B.K.C., male, æt. 35, was admitted into Brahmachari's ward in the Chittaranjan Hospital, with history of fever for three months. The spleen was hard and extended $4\frac{1}{2}$ in. and liver extended 2 in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given eight injections of the compound intramuscularly in doses of 0·1 to 0·2 gramme, at first twice a week and subsequently every other day. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen and liver could not be felt below the costal arch.

*Total dose*—1·4 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—2,200,000 ; W.B.C.—1,550 ; Hb.—45 per cent.

After treatment : R.B.C.—30,00,000 ; W.B.C.—7,800 ; Hb.—55 per cent.



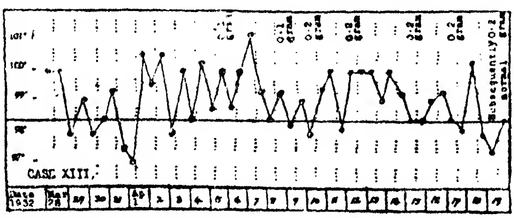
*Case XII.*—A.M., male, æt. 26, was admitted into the Chittaranjan Hospital, with history of fever for six months. The spleen was hard and extended 4 in. below costal margin. Liver was not palpable. Leishman-Donovan bodies were found on spleen puncture. Patient was given nine injections of the compound intramuscularly in doses of 0·05 to 0·4 gramme, at first twice a week and subsequently every other day. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen could not be felt below the costal arch.

*Total dose*—1·95 grammes.

*Result of Blood Examination*—

Before treatment: R.B.C.—1,300,000; W.B.C.—1,800; Hb.—35 per cent.

After treatment: R.B.C.—1,700,000; W.B.C.—6,000; Hb.—45 per cent.



*Case XIII.*—M.N.M., male, æt. 20, was admitted into the Chittaranjan Hospital, with history of fever for some months. The spleen was hard and extended 4 in. below the costal margin. Liver was not palpable. Leishman-Donovan bodies were found on spleen puncture. Patient was given fifteen injections of the compound intramus-

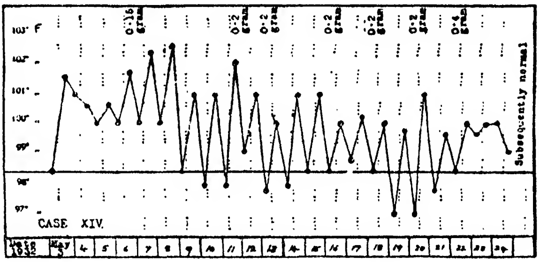
cularly in doses of 0·1 to 0·4 gramme, generally every other day and sometimes twice a week. Effect of treatment on temperature is shown in the accompanying Chart. At the time of discharge, spleen could just be felt below the costal arch.

*Total dose*=2·85 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—1,700,000 ; W.B.C.—1,550; Hb.—35 per cent.

After treatment : R.B.C.—2,000,000; W.B.C.—5,700; Hb.—50 per cent.




*Case XIV*.—U., male, æt. 35, was admitted into the Chittaranjan Hospital, with history of fever for six months. The spleen was hard and extended 6 in. and the liver extended 2 in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given nine injections of the compound intramuscularly in doses of 0·1 to 0·4 gramme, at first twice a week and subsequently every other day. Effect of treatment on temperature is shown in the accompanying Chart. After completion of treatment, spleen and liver could not be felt below the costal arch. Patient is now under observation in the hospital.

*Total dose*—1·8 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—2,300,000 ; W.B.C.—1,550; Hb.—50 per cent.

After treatment : R.B.C.—3,000,000 ; W.B.C.—5,744 ; Hb.—65 per cent.

*Case XV.*—K.P.M., male, æt. 15, was admitted into the Carmichael Medical College Hospitals, with history of fever for seven months. The spleen was hard and extended 7 in. and liver extended 4 in. below the costal margin. Leishman-Donovan bodies were found on spleen puncture. Patient was given ten injections of the compound intramuscularly in doses of 0·05 to 0·3 gramme twice a week. At the time of writing this paper, the spleen extended 3 in. below the costal margin and the patient was still under treatment.

*Temperature*—Apyrexial throughout the course of treatment.

*Total dose*—1·9 grammes.

*Result of Blood Examination*—

Before treatment : R.B.C.—2,500,000 ; W.B.C.—2,500; Hb.—40 per cent.

At the time of writing : R.B.C.—2,520,000 ; W.B.C.—3,500 ; Hb.—60 per cent.

## Observations

The toxicity of sodium-sulphomethyl-stibanilate is low. Its maximum tolerated dose is 0·4 per kilo. of body weight in the case of white rats given intravenously. It has been successfully used in the treatment of kala-azar by intramuscular injection. Generally speaking no local or constitutional symptoms have been observed after its use. It has been injected up to a dose of 0·4 gramme. One injection of 0·5 gramme was given to one patient without any untoward results. In one case complicated with nephritis, œdema, and epistaxis, no untoward results followed its use. Originally, the compound was used in doses of 0·1 to 0·2 gramme intramuscularly, but it has been observed that the dosage

can be increased in an adult from 0·2 to 0 4 gramme without any constitutional symptoms. It has been used every other day in some cases and twice a week in others.

Other antimony compounds previously used intramuscularly in the treatment of kala-azar have been dealt with in an earlier paper by the author and co-workers and therefore need not be mentioned here.

[*Reprinted from the Indian Medical Gazette, Vol. XLI, January, 1906*]

# FORMS OF PYREXIA DUE TO LEISHMAN-DONOVAN BODIES

(1) High intermittent pyrexia :—

Fever may or may not be attended with rigors. (Chart No. I.)

(2) Irregular intermittent pyrexia :—

This type of fever is commonly seen. The fever may or may not be attended with rigors. (Chart No. II.)

(3) Double quotidian pyrexia with double intermission during 24 hours :—

There is rise of temperature towards very early morning followed by intermission before noon. There is a second rise in the evening followed by intermission before midnight. (Chart No. III.)

(4) Double quotidian pyrexia with single intermission during 24 hours :—

There is a rise of temperature towards very early morning followed by intermission as in the above. There is a second rise in the evening which is followed by remission and not intermission before midnight. (Chart No. IV.)

(5) Intermittent pyrexia with irregular periods of apyrexia.

(6) Remittent pyrexia.

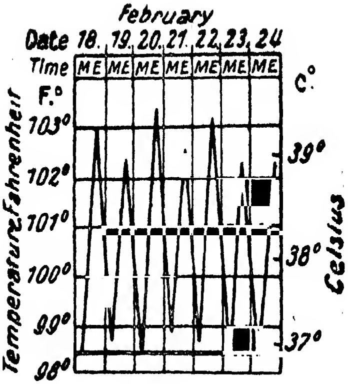



Chart 1.

Temperature chart of a kala-azar case showing high intermittent pyrexia.

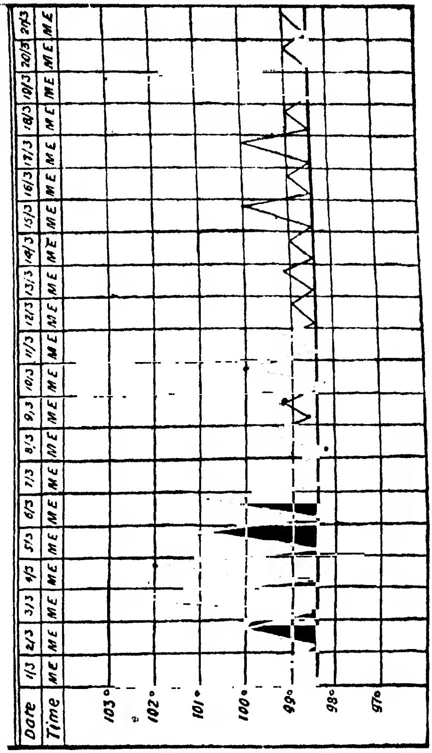



Chart 2.

Temperature chart of a case showing irregular intermittent pyrexia.

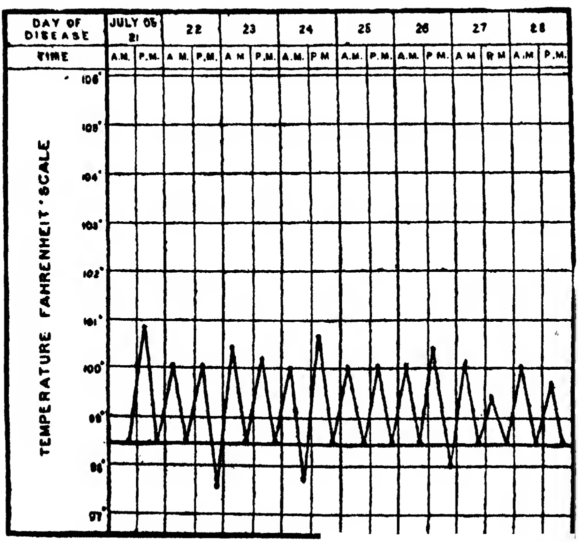



Chart 3.

Temperature chart of a kala-azar case showing Double quotidian pyrexia with double intermissions during 24 hours.

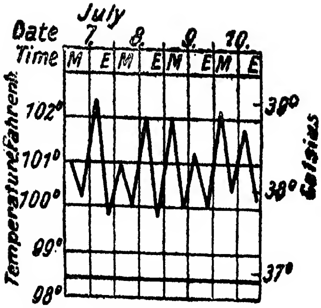
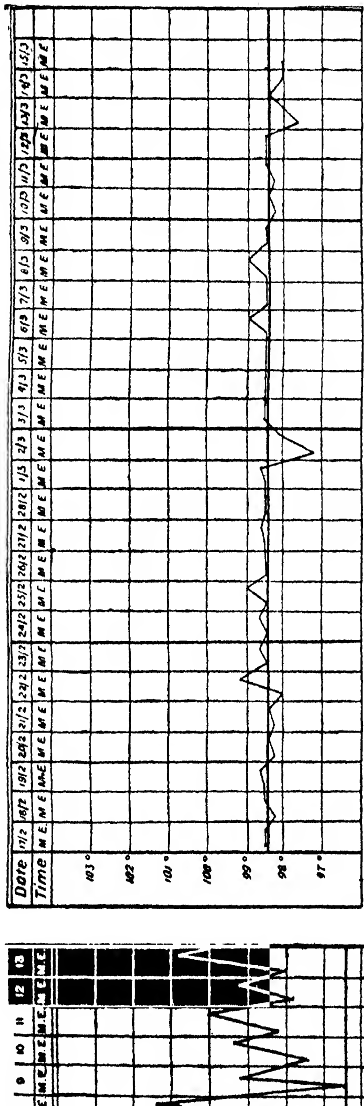
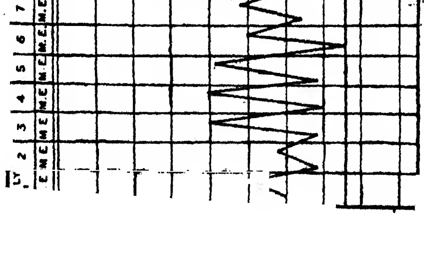



Chart 5.

Temperature chart of a kala-azar case showing Double remittent pyrexia.





(7) Double remittent pyrexia :—

There is a rise of temperature towards very early morning followed by remission before noon. There is a second rise in the evening followed again by remission before midnight. The temperature does not come down to normal. (Chart No. V.)

(8) Combined intermittent and remittent pyrexia resembling hectic :—

Patients may have this type of temperature for a long time. (Chart No. VI.)

(9) The presence of Leishman-Donovan bodies is not necessarily associated with much pyrexia. (Chart No. VII.) There may be very slight rise of temperature for some time. *

The great peculiarity of the pyreixa due to the Leishman-Donovan bodies is its variable nature. The various types may be combined in one and the same patient. There was a case who had at first low intermittent fever for some time, then high intermittent fever and then remittent fever. No explanation has as yet been offered of this variability of the temperature curve. There may also be variable periods of apyrexia in the course of the disease, though the parasites may still be present in the spleen. The double remittent type of pyrexia may pass into the simple remittent type.

* Triple quotidian pyrexia has also been noted in kala-azar and sometimes the pyrexia may resemble that of pyæmia.—*Ed.*]

[*Reprinted from the Calcutta Medical Journal, October, 1906*]

# A CONTRIBUTION TO THE STUDY OF FEVERS DUE TO LEISHMAN-DONOVAN BODIES *

The discovery of the Leishman-Donovan bodies in the blood cf the spleen of patients suffering from what used to be called "malarial cachexia" has revolutionized our ideas about the causation of this disease. Until very recently, all cases of enlarged spleen with anæmia, wasting and a history of fever resembling that of true malaria and coming from more or less malarious places, used to be called "malarial cachexia." Six years ago, while studying the malarial fevers in the Calcutta Medical College Hospitals, I used to find a number of cases with enlarged spleen, in whose blood no malarial parasites were found. Still one used to call such cases malarial, because their resemblance to malaria was great, though at the same time differing from it in not being amenable to quinine or only slightly so. It is, I think, this confusion of malarial fevers with those due to the Leishman-Donovan bodies, that has led many physicians in Bengal to believe that there are two kinds of malarial fever, one amenable to quinine and the other not. Cases of true malarial fever not yielding to quinine given in sufficient doses and for a sufficient length of time must be rare. Of course there are cases in which quinine may not be absorbed from the gastro-intestinal tract, but such cases are far from being common. One such case I shall cite to you. A girl, about 7 years old, was treated by me at Dacca, for intermittent

* Read at the Calcutta Medical Club, 6th September, 1906

fever coming on with ague every day, with slightly enlarged spleen and a tender liver. Her blood showed the presence of malignant tertain parasites. She was given large doses of quinine but there was no disappearance of the fever nor were there any symptoms of quininism. Though quinine was given in solution still I believed that it was not absorbed into the system and my suspicion was corroborated by my being able to detect its presence in the fæces. This fact is not strange because we know that in another disease *viz.*, cholera, medicines are not absorbed and may remain inside the stomach unabsorbed for days together and then show suddenly poisonous symptoms in the stage of re-action. Leaving, however, these exceptional cases in which quinine is not absorbed into the system, we may say almost with certainty that one of the surest tests for malarial fever is its amenability to quinine. This fact has been amply proved by the discovery of the Leishman-Donovan parasites, which are responsible for a large number of cases of fever simulating malaria. These latter have resisted quinine giving it the bad reputation of not being infallible in the treatment of malaria.

When I first began to study cases of malarial cachexia in the Calcutta Medical College Hospitals it struck me that such cases were much more common and had a much worse prognosis in India than in America. I used to explain to myself their more frequent occurrence being due to less frequent use of quinine in India. But when I found that they never showed any malarial parasites in the blood, that their fever could not be checked by quinine, that a very large number of them ended fatally and many of them had *cancrum oris* and dropsy it struck me that their cause was something that was not known to us.

The fever due to Leishman-Donovan bodies has been termed cachexial fever or cachectic fever by Major Leonard Rogers. But as the disease may exist for a variable period without any fever, I think it may be more appropriately

called leishman-donovan disease or tropical cachexial disease, a term though cumbrous is perhaps more accurate.

It has not as yet been worked out how the parasites enter the system. The disease is frequently found in places where true malarial fevers occur. It is probably propagated through water or through the mosquito or the parasite may enter the system through the gastro-intestinal tract.

As regards the early *symptoms* of the disease our knowledge is still very limited. The cases that we meet with in the hospital wards are those that have suffered for a long time. I give here a summary of the early symptoms of the disease as gathered from the patients.

In *one class* of cases we get a history of intermittant attacks of fever, quotidian for some time and later on becoming irregular with or without rigors, lasting for several months and not benefited or only temporarily benefited by quinine. In a *second class* there is no history of any previous attacks of fever but the spleen becomes slowly and steadily enlarged. In a *third class* there is a history of attacks of low fever, continued for several months with a progressive enlargement of the spleen and not benefited by any treatment. In a *fourth class* there is history of one or two attacks of remittent fever more or less resembling typhoid. In a *fifth class* the patient gives history of gastro-intestinal troubles with dysenteric or diarrhœic attacks followed by œdema of the lower extremities and attended from time to time with ague-like attacks of fever.

In all these cases, the patient invariably says that there was very slight or no benefit from quinine.

One of the most acute and fatal cases that I have ever seen was a child whom I treated some time ago. She was about 4 years old, belonged to a rich family and was brought back to Calcutta from Eastern Bengal where she had been taken to from Calcutta and kept about a month in a malarious district. She was suffering with high remittent

fever lasting for about a week. She had *cancrum oris* on the 4th day of the fever. When I examined her, I found her anæmic, with a large portion of the left cheek gangrenous and with slight enlargement of the spleen and slight bronchitis. The fever was of a remittent type. The *cancrum oris* spread very quickly and the patient died five days after being brought to Calcutta. I might remark here, that I had seen the child shortly before her leaving Calcutta, in a perfectly healthy condition.

I have observed eight different types of pyrexia in this disease. These were published in the *Indian Medical Gazette*, January, 1906. [They will not be reproduced here.—*Ed.*]

The great peculiarity of pyrexia in this disease is its variable nature. As shown before, the various types may be combined in one and the same patient. No explanation has as yet been offered of this variability of the temperature curve. Due to some unknown cause the temperature of the patient may remain perfectly normal for several days or weeks though the parasites may be seen in large numbers in the spleen.

A very common symptom in this disease is œdema of the lower extremities. It is sometimes an early symptom of the disease. On the other hand it may be absent even up to the last. It is not easy to explain the cause of the œdema. The œdema is not due simply to anæmia because sometimes it is a very early symptom of the disease and then we would notice it more markedly in true malarial cachexia which is attended, as Major Rogers has pointed out, "with more anæmia" than cachexial fever.

I show you here the photographs of two cases taken a few days before death, one of whom had a very well marked œdema and the other, almost none at all. Both were advanced cases being anæmic, having no albumen

in the urine and having no abnormality in the heart. It has struck me that we can differentiate the disease into two classes, one in which œdema is a marked symptom and the order in which it is least marked. This would remind one of the two types of beriberi that have been observed, the wet and the dry varieties. Cases in which œdema of the lower extremities is completely absent is more rare than those in which it is present.

Gastro-intestinal symptoms are also often noticed in this disease. Attacks of dysentery or diarrhœa are often present and sometimes there may be a most intractable attack of dysentery which may end fatally.

Wasting and anæmia are marked symptoms of the disease. There is a peculiar facies which enables one to suspect the disease in many cases.

The spleen is enlarged in all my cases but it is not necessarily very large or very hard. In some of the worst cases it did not extend beyond 3″ below the costal arch. In most of the cases the spleen is moderately enlarged. In some very large and hard spleens I did not find any Leishman-Donovan bodies. I have often been led to suspect the disease when I found moderately enlarged spleen with extreme emaciation, cachexia and œdema of the lower extremities, symptoms which we rarely meet with in cases of malaria with moderately enlarged spleen. The liver is enlarged in a large proportion of cases. Sometimes the patients complain of pain over the splenic region which in some cases at least might be due to infarcts, often seen in this disease.

*Complications*—(1) Pneumonia, (2) Phthisis, (3) Dysentery, (4) Diarrhœa, (5) *Cancrum oris*, (6) Hæmorrhage from the gums or under the skin in advanced cases, (7) Delirium or coma in some cases, shortly before death, (8) Albumen in the urine in a few cases, which might be due to a

concomitant kidney disease. I have not met with a case in which there was perforation of the bowels as has been noted by some observers.

*Diagnosis.*—The surest sign is the presence of the Leishman-Donovan bodies in the blood of the spleen. Spleen puncture is a simple operation and I have not met with a single accident. In some cases the patient complain of pain and tenderness for a day or two at the point of puncture. Rarely there is a more general tenderness. I have been more fortunate than some other observers, who have met with fatal cases of hæmorrhage after spleen puncture. In one of my cases, with hæmorrhage from the gums, hæmorrhage under the skin and marked œdema of the lower extremities, spleen puncture was not accompanied with any untoward results except that the patient complained of pain for 3 or 4 days. Hæmorrhage to a great extent depends, I think, on the size of the needle used. I use the long needles supplied by B. W. & Co., for intra-muscular injections. I puncture the spleen at a part in intimate contact with the abdominal wall, press with a finger at the punctured spot for about 5 minutes and immediately put the patient on calcium chloride or turpentine. In bad cases I put the patient on calcium chloride for 2 days before spleen puncture. One or two drops of blood drawn from the spleen are enough to demonstrate the presence of the parasites if they are present. While therefore admitting the possibility of the danger of spleen puncture as pointed out by other observers, I think we may reduce it to a minimum if we do not use large needles and draw not more than a few drops of blood.

I have observed the parasites *post-mortem* in the splenic veins, the liver, the portal veins and the bone marrow. I have never found them in the superior or inferior vena cava. In one case I found the Leishman-Donovan bodies

in the blood inside the heart within a leucocyte as will be seen in the accompanying Diagram. The L.D. bodies are very rarely found inside the red blood corpuscles. Dr. Christophers observes that he never saw unmistakable forms in red cells either in peripheral or splenic blood. In one case, however, I have observed the bodies inside red cells in the splenic blood, apparently indicating that the parasites may infect the red blood corpuscles like the malarial parasites.*

All cases of enlarged spleen met with in India may not be due to Leishman-Donovan disease or malaria. Some of them may be due to some other micro-organism. Others may be due to a hitherto unknown phase of *Leishmania donovani*.

Major Rogers lays great stress on leucocyte count as a method of diagnosis in this disease.

Double remittent pyrexia and double quotidian pyrexia with double or single intermission in 24 hours, have been considered very suspicious of this disease but I do not consider them to be absolutely pathognomonic. On the other hand we may say that a fever which is at first intermittent in type and shows from time to time double rise with single or double intermissions in 24 hours or passes into a remittent type which also tends to show double rise, and is spontaneously attended with variable periods of apyrexia and associated with enlargement of the spleen, general wasting and œdema of the lower extremities should be regarded as very suspicious of the disease due to Leishman-Donovan bodies.

In the Scientific Memoirs for 1905 Captain James lays stress upon the following group of signs and symptoms as very significant of true malarial cachexia: (1) Enlargement

* It has now been conclusively proved that Leishman Donovan bodies never infect the red blood corpuscles and that the so-called leishmania-infected red cells are mere artifacts.—*Ed*

of spleen, (2) a temperature-curve which shows definite pyrexial and apyrexial periods and (3) absence of serious symptoms throughout the period during which the condition lasts, but especially so during the apyrexial intervals. Cases of Leishman-Donovan disease in which the temperature curve shows pyrexial and apyrexial periods are not uncommon. In some of my cases I have noticed periods of apyrexia lasting for several days. The temperature Charts were very carefully drawn, the temperature being taken invariably every 4 hours and in some cases even every 2 hours. On the other hand there are many undoubted cases of true malarial cachexia in which the condition becomes progressively worse if untreated. I cannot therefore regard the above group of symptoms as absolutely pathognomonic of malarial cachexia.

*Prognosis* is always grave. A large number of my cases died. Some left the hospital in a worse condition than at the time of admission. None of my cases was benefited by treatment. Perhaps some do get spontaneously cured. It is impossible in the present state of our knowledge to give a prognosis of the very early cases. Captain James believes that the tendency of true malaria is to eradicate itself from the system, instead of a cachexia setting in. He holds that immunity to malaria may be acquired by repeated attacks. There can be little doubt that some malarial cases do get progressively worse without acquiring immunity. Thus it would appear that the prognosis of many cases of true malarial cachexia may be unfavourable, unless properly treated.

*Treatment.*—No drug can check the progress of this disease. I have tried quinine in small and very large doses, by the mouth or by hypodermic injection, without any good result. In some cases it temporarily reduces the temperature to a slight extent. Fluorides and arsenic have been tried without any effect. Cacodylate of sodium

and arrhenal seemed to do some good, though only temporarily. The latter drug was also injected in $\frac{1}{2}$ gr. doses into the spleen. Methylene blue, hetol and nuclein have been tried without success. In two cases I injected methylene blue into the spleen without any untoward results. An intercurrent attack of *cancrum oris* or a large abscess may lead to a cure.

# TRANSACTIONS OF THE CALCUTTA MEDICAL CLUB

(ABSTRACT)

On the 24th June, 1906, Dr. Upendranath Brahmachari read a paper on "A Contribution to the Study of Fevers due to Leishman-Donovan bodies" (*vide* the Calcutta Medical Journal, October, 1906). Dr. Kailaschandra Bose presided.

Dr. Satyasaran Chakravarti said that although quinine test was believed to be infallible in cases of malaria by many authorities, cases sometimes cropped up which showed malarial parasites but which did not yield to quinine. One such case was lately published in the I. M. G.

There are cases which clinically are indistinguishable from malaria and in some cases there may be true mixed infections, although authorities differ on this point. Diagnosis rests on spleen puncture and demonstrating Leishman-Donovan bodies in the splenic blood and although the reader of the paper is very fortunate, this little operation has sometimes been attended with death. Spleen puncture is not safe specially in a country where spontaneous rupture of the spleen is not rare. Therefore, would it not be wise to trust to peripheral blood counting in at least those cases where spleen puncture would seem at all dangerous? It is desirable, when the operation is done to take such precautions as giving calcium chloride beforehand and determining the coagulability of the blood in each case. As regards the operation itself perfect asepsis, straight puncture without side movement, pressure after the operation and rest in bed are better enjoined. Everything, I venture to say, does not depend on the size of the needle. Of course, the finer the needle the less the chance of hæmorrhage, but more the

difficulty of getting sufficient blood. A larger amount is necessary when one wants to culture the organism. The anatomical state of the spleen to be punctured should be taken into account as well. In a suspicious case, a peripheral blood count when it shows a diminution both in the red and white corpuscles, marked diminution in the number of the white blood corpuscles, especially of the polymorphonuclear variety, together with an increase in the large mononuclear and the lymphocytes, the case is decidedly associated with Leishman-Donovan bodies in the splenic blood. This should be a sufficient guide in diagnosis when spleen puncture would seem dangerous. The cure of cachexial fever after *cancrum oris* attributed to the development of staphylococcus toxin is doubtful. Any marked leucocytosis brought on by any means tends to cure this disease. How often cultivators from villages suffering from other complaints are seen in the wards with huge hard spleens and many blister or actual cautery marks over the abdominal wall who are sufficiently healthy to be able to do their work. They are fairly robust and free from fever. These I take to be cases of cachexial fever cured by the leucocytosis brought about by repeated cautery or blister. About the prognosis of the condition though it has been said that the cases are invariably fatal, there are authorities who hold that it is not necessarily always so. According to the latter, if the patient eats well and has a good digestion and if he can be kept going on for 18 months he recovers.

As Dr. Brahmachari says, other forms of enlarged spleen may hereafter be shown to be due to other organisms or to some unknown phase of the parasite other than the Leishman-Donovan bodies. The presence of Leishman-Donovan bodies has been demonstrated in the peripheral blood by Donovan though not found by others. As regards treatment, though it is not very encouraging, it is not so gloomy as Dr. Brahmachari would lead one to believe. Quinine though useless may prove to be valuable after the anæmia has been suitably treated for some time but certainly local puncture of the splenic region and injection of quinine have been found to be useful in a certain number of cases in which the organism was found. In laboratory animals experimental inoculation of blood containing trypanosoma has failed to infect them, if the animals were previously treated with trypan red or malachite green.

Horses suffering from trypanosoma infection have been cured by these drugs according to Koch. These might, in suitable doses, be useful in man.

Owing to the lateness of the hour the meeting was adjourned.

The adjourned meeting was held in the premises of the club, on Saturday, the 26th June, Dr. Kailaschandra Bose, President, in the chair.

The further discussion on Dr. Brahmachari's paper was resumed.

Dr. Rajendralal Dey spoke of his experience about this disease. All the cases treated by him ended fatally. He suggested the use of X-ray.

Dr. Haridhan Datta thought that the early symptoms simulated those of typhoid fever. The type of the disease with no fever and sudden enlargement of the spleen, was very rare. A slight evening rise of the temperature may not be noticed by the patient. Quinine was useless but might help in diagnosis. The different forms of pyrexia, alluded to in the paper, made the diagnosis more difficult. He did not believe that a good many cases diagnosed as remittent fever were really cases of Leishman-Donovan disease. He thought that most of the cases seen in Calcutta were imported from outside. He could not explain why the temperature should be so variable. He objected to spleen puncture for diagnostic purposes and thought that practitioners were not justified to have recourse to untried methods in private practice. He did not believe in the recovery of early cases or in spontaneous cure. He has tried guaiacol in some cases and found it to be useful. The theory of the propagation of the disease by bugs was untenable.

Dr. Sashibhushan Mukherji spoke about the difficulty in diagnosis. He advocated a change of climate and drugs to improve the general health.

Dr. K. G. Sircar thought that failure of quinine in checking fever did not imply that the fever was not malarial. He referred to Dr. Crombie's remarks about unclassified malarial fevers of India. He related a case in which repeated blood examination showed nothing. The patient was a Eurasian adult male, residing in Calcutta. He first saw the patient after a month's slow fever with an enlarged spleen. Large doses of quinine had no effect. A change to Darjeeling did him no good and the patient came back to Calcutta

much worse. A prolonged treatment with small doses of quinine, arsenic and iodine, with occasional cacodylate injections cured him.

Dr. Purnachandra Nandi thought that quinine might be efficacious if combined with iron.

Dr. Balaichandra Sen mentioned a case in which no positive result was obtained by a blood examination. He thought that quinine would be useless in those cases where the liver was enlarged. Acid treatment might be efficacious.

Dr. Kedarnath Das thought that prognosis would be better if we could bring about increased leucocytosis by some means.

Dr. Upendranath Brahmachari, in reply, said that the failure of quinine in malaria (proved by a blood examination) is almost unknown. Such cases were generally complicated with the other disease, thus explaining the failure of quinine test. He did not think that leucocyte count, as helping diagnosis, was always satisfactory. As complications were common in Leishman-Donovan disease, the result of leucocyte count was bound to be vitiated. In children leucocyte count was always doubtful.

The president observed that the effect of quinine depended on the way it was exhibited. Quinine test was not always diagnostic. The mere presence of malarial parasites in the blood did not indicate that Leishman-Donovan bodies were absent. Both may exist together. He did not agree with the lecturer that spleen puncture was safe.

[Reprinted from the British Medical Journal, May, 1908]

# SPORADIC KALA-AZAR IN CALCUTTA, WITH NOTES OF A CASE TREATED WITH ATOXYL

Kala-azar, as used in this article, may be defined as the disease caused by the Leishman-Donovan bodies. Its epidemic manifestation, which is seen in Assam, more frequently goes by this name. It is endemic round about Calcutta and probably occurs in the city itself.

Last year, I examined the spleen blood of nearly 150 cases of enlarged spleen admitted into my wards in the Campbell Hospital, and found the parasite of kala-azar in 60 cases. From this it is seen that cases of this disease are frequently admitted into the Calcutta hospitals.

No two cases came from the same house, and I have not succeeded in corroborating the fact that the disease is limited to individual houses or families; 91·7 per cent of my cases were Hindus, while 8·3 were Mahomedans, giving a proportion of nearly 11 to 1. Out of the total number of patients admitted into my wards in 1907, about 75 per cent were Hindus and 17·5 per cent Mahomedans, giving a proportion of nearly 4 to 1. It will thus be seen that Hindus are more frequently affected by the disease than the Mahomedans, and if this fact is corroborated by more extended observations it may probably throw some light on the etiology of the disease. Thirty-three per cent of my cases were below the age of 20. I have not met with any case above the age of 35. The cases came to my wards during all the parts of the year, the largest number of admissions being

during the period of April to August, but especially in the month of May.

It was difficult to trace from the history of the cases where the disease was contracted. Many of them pointed, however, to the disease having been contracted in the neighbourhood of Calcutta, and a few seemed to have contracted the disease in the city itself, as they never went out of it. None of my cases came from the epidemic area of Assam. Some came from Orissa, Eastern Bengal, district of Murshidabad, and Behar.

Most of my cases were chronic, with history of illness for several months, and with the spleen extending 3 inches or more below the costal arch. In a large majority the liver was also moderately enlarged, while in a very few cases the liver was very much more enlarged than the spleen, which extended just an inch or so below the ribs. I have not met with a case in which the spleen was not at all enlarged.

## SYMPTOMS

As regards the early symptoms of the disease our knowledge is still very limited. The patients met with in the hospital wards have suffered for a long time. I give here the summary of the early symptoms of the disease as gathered from the patients. (*Vide* Indian Medical Gazette, Vol. XLI, January, 1906.)

In *one class* of cases we get a history of intermittent attacks of fever, quotidian for some time, and later on becoming irregular with or without rigors, lasting for several months and not benefited or partly benefited by quinine. In a *second class* there is a history of a few previous attacks of fever, continuing for some time on each occasion, the spleen becoming slowly and steadily enlarged. In a *third class* there is history of attacks of low fever, continued for several months, with a progressive enlargement of the spleen not benefited by any treatment. In a *fourth class*

there is a history of one or two attacks of remittent fever more or less resembling typhoid. In a *fifth class* the patient gives a history of gastro-intestinal troubles, with dysenteric or diarrhœa attacks followed by œdema of the lower extremeties and attended from time to time with ague-like attacks of fever. In all these cases the patient invariably says that there was very slight or no benefit from quinine. Possibly a large majority of cases begin with attacks of remittent fever.

Among the other symptoms that I have observed may be mentioned progressive emaciation, anæmia, cachexia, œdema of the extremeties, diarrhœa, dysentery, which may sometimes be very obstinate, and hæmorrhage from various parts of the body, such as the skin and the mucous membranes.

Œdema of the extremities is sometimes an early symptom. On the other hand, it may be absent even up to the last. It is not easy to explain the cause of this œdema. It appears that we can distinguish two classes of cases, one in which œdema is a marked symptom and the other in which it is not.

I have elsewhere described in detail the various types of pyrexia that I have observed in this disease (Indian Medical Gazette, January, 1906). Besides these, there may sometimes be pyrexia of the pyæmic type, there being more than two remissions or intermissions in twenty-four hours. Rarely the disease takes on an *apyretic* course for an indefinite length of time.

## Blood Count

In the main my observations corroborate those of Major Rogers (Lancet, March 2nd, 1907). Leukopenia is extreme and in many cases the proportion of white to red cells is less than 1 :1,000 (Table I).

TABLE I

| | Red Corpuscles. | White Corpuscles. | Proportion. |
|---|---|---|---|
| 1 | 2,390,000 | 1,720 | 1/1389 |
| 2 | 2,530,000 | 2,530 | 1/1000 |
| 3 | 2,740,000 | 1,792 | 1/1529 |
| 4 | 3,440,000 | 2,386 | 1/1441 |
| 5 | 3,170,000 | 2,380 | 1/1332 |
| 6 | 2,660,000 | 1,160 | 1/2293 |
| 7 | 2,760,000 | 1,527 | 1/1807 |
| 8 | 2,920,000 | 1,320 | 1/2212 |
| 9 | 2,120,000 | 1,736 | 1/1221 |
| 10 | 2,330,000 | 1,600 | 1/1456 |

In a number of cases, though there was a marked leukopenia, yet the proportion of white to red was higher than $\frac{1}{1000}$. There are probably very advanced cases with extreme anæmia (Table II).

TABLE II

| | Red Corpuscles. | White Corpuscles. | Proportion. |
|---|---|---|---|
| 1 | 1,400,000 | 2,170 | 1/645 |
| 2 | 860,000 | 2,250 | 1/382 |
| 3 | 1,480,000 | 3,870 | 1/382 |
| 4 | 1,300,000 | 3,300 | 1/383 |

In many cases complications often lead to increase in the leucocyte count (Table III).

TABLE III

| | Red Corpuscles. | White Corpuscles | Proportion. | Complication. |
|---|---|---|---|---|
| 1 | 2,880,000 | 7,662 | 1/376 | Pneumonia. |
| 2 | 896,000 | 2,768 | 1/323 | *Cancrum oris*. |
| 3 | 3,070,000 | 3,500 | 1/817 | 24 hours after application of a large blister over the splenic region. |

Lastly, cases that have been recovering or have recovered from *cancrum oris*, or have been treated with atoxyl for some time may give a higher relative leucocyte count than 1/1,000 (Table IV).

TABLE IV

| | Red Corpuscles | White Corpuscles. | Proportion | Complication. |
|---|---|---|---|---|
| 1 | 2,340,000 | 4,569 | 1/512 | Recovery from cancrum oris. |
| 2 | 2,600,000 | 3,340 | 1/778 | Treatment with atoxyl. |
| 3 | 3,440,000 | 5,060 | 1/529 | Do |

Comparing the leucocyte count in kala-azar with what we observe in malarial cachexia, exactly the same conclusion as that of Major Rogers is reached, namely, that a ratio of red to white corpuscles below 1/1,500 is almost diagnostic of kala-azar ; but, as we have shown above, we may meet with cases without any apparent complication, or some with well-marked complications, or others that have taken a favourable turn, in which the proportion may be higher than 1/1,000.

I append here a table showing the blood count in malarial cachexia in which the proportion of white to red is much greater than 1/1,000 (Table V).

TABLE V

*Malarial Cachexia*

| | Red Corpuscles. | White Corpuscles. | Proportion. |
|---|---|---|---|
| 1 | 3,240,000 | 8,000 | 1/405 |
| 2 | 2,208,000 | 3,372 | 1/655 |
| 3 | 2,384,000 | 4,863 | 1/490 |

## COMPLICATIONS

(1) Pneumonia ; (2) phthisis ; (3) dysentery ; (4) diarrhœa; (5) *cancrum oris* and its attendant complications; (6) hæmorrhage from the gums or under the skin in advanced cases ; (7) delirium or coma, in some cases, shortly before death ; (8) albumen in the urine in a few cases, which might be due to concomitant kidney disease ; (9) paraphimosis ; (10) hæmoptysis ; (11) hæmatemesis ; (12) melæna ; (13) epistaxis ; (14) œdema of the extremeties; (15) splenalgia due to infarcts in the spleen ; (16) hæmorrhoids, which may bleed obstinately and profusely; (17) large abcesses. I have not met with a case in which there was perforation of the bowels, which Captain Christophers describes in some of his cases.

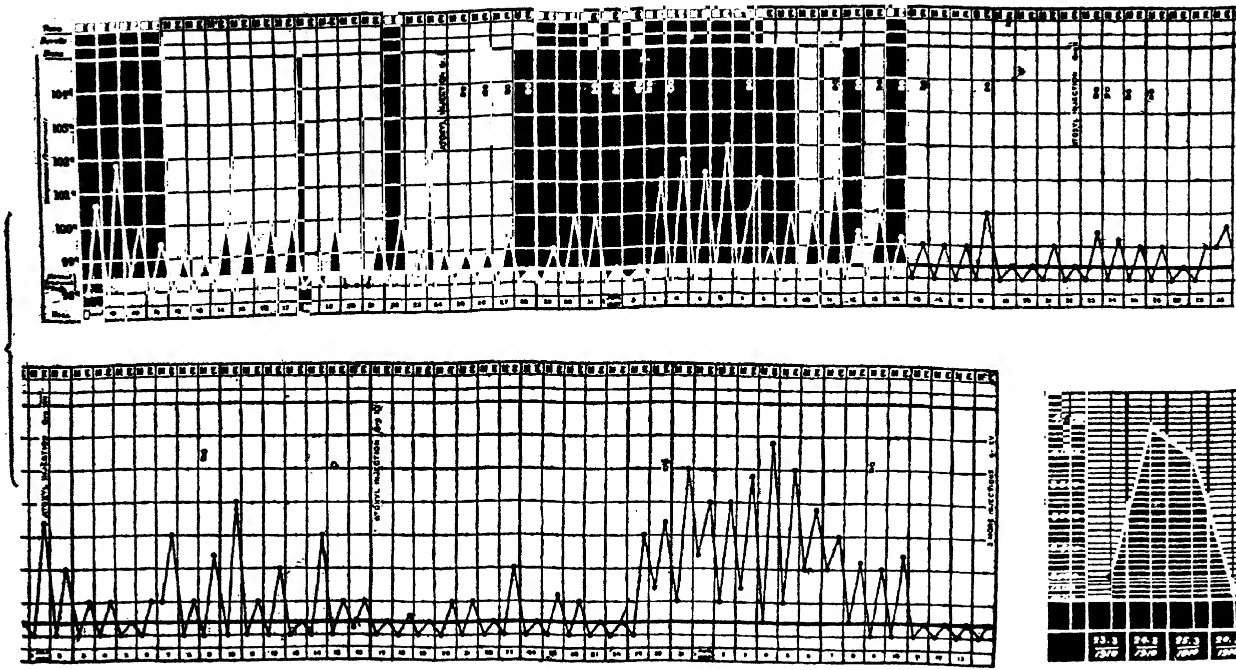
## PROGNOSIS

According to my observations, the prognosis is always grave. I have not the record of a single case which I could pronounce cured; 40 per cent. of my cases died in the hospital, the most frequent cause of death being intractable diarrhœa or dysentery.

## TREATMENT

So far as my observations go, no drug can kill the parasites. I have used the following drugs without success :

Temperature chart of a kala-azar case treated with atoxyl. (Para. 3).



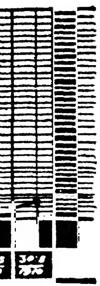


Chart showing effect of hetol on leucocyte count.

(1) quinine internally; (2) quinine hypodermically; (3) fluorides; (4) arsenic and some of its new preparations, such as sodi cacodylate, arrhenal, and atoxyl; in some cases arrhenal and sodi cacodylate were given hypodermically; (5) methylene blue internally; (6) methylene blue hypodermically; (7) izal in increasing doses; (8) cyllin in increasing doses up to half a drachm thrice a day.

Encouraged by the results of the use of atoxyl in trypanosomiasis, I gave it an extensive trial in a few cases, one of which has been under my observation for nearly eight months and under atoxyl treatment for nearly six months. As far as I am aware, there are no records of a single case in which atoxyl was used for such a prolonged period in kala-azar.

The patient, æt. 30, a Hindu, had slight œdema of the lower extremities at the time of admission, the spleen extending 8 in. from the left nipple to the middle line.

The effects of the treatment were as follows:

1. Body Weight—

| | | St. | lb. |
|---|---|---|---|
| May 25, 1907 | ... | 6 | 0 |
| August 12, 1907 | ... | 7 | 4 |
| September 9, 1907 | ... | 7 | 5½ |
| November 16, 1907 | ... | 8 | 0 |
| December 29, 1907 | ... | 8 | 5½ |

2. Blood Count—

| Date. | Red Corpuscles | White Corpuscles. | Proportion. |
|---|---|---|---|
| May 2, 1907 | 2,760,000 | 1,527 | 1/1807 |
| August 16, 1907 | 2,500,000 | 1,600 | 1/1562 |
| September 7, 1907 | 2,280,000 | 1,950 | 1/1170 |
| November 18, 1907 | 2,600,000 | 3,340 | 1/778 |
| January 7, 1908 | 3,440,000 | 6,500 | 1/529 |

3. Pyrexia—(There is some effect on the Temperature curve.)

4. General condition—The patient looks much better in health; the œdema of the extremities has completely disappeared. He is stouter than before, is less anæmic, is not cachectic and has got better appetite.

5. Toxic action—The drug was almost non-irritating. Except on one occasion there was very slight local irritation. On this occasion there was a slough which I think was due to the solution being too hot. In other cases in which I have given injection of atoxyl no untoward local symptoms were met with.

6. Effect on the parasites—They are still found in the splenic blood. Some of them present a granular appearance, but they are still to be seen in large numbers.

7. Spleen—Not much diminished in size.

Before concluding, I would just mention one point, that spleen puncture, though it has been pronounced dangerous by the highest authorities, has never led to any single accident in any of my cases. I have found that a large hypodermic needle is sufficient to enable one to draw one or two drops of blood, which are almost always sufficient to show the parasites if they are present. I would therefore recommend that not more than one or two drops of blood should be drawn from the spleen for ordinary diagnostic purposes. It appears to me that the danger of spleen puncture may be reduced to a minimum if one uses a hypodermic needle, gives the patient calcium chloride before and after puncture, enjoins absolute rest in bed, and puts pressure upon the punctured spot for about fifteen minutes after the operation.

## Conclusion

1. Sporadic kala-azar is frequently seen in Calcutta hospitals and is probably endemic in Calcutta itself. Hindus are probably more affected than Mahomedans.

2. The leucocyte count is of great diagnostic help but not absolutely so in kala-azar.

3. Atoxyl is borne in very large doses (grs. xv in one injection) by kala-azar patients. It is perfectly harmless and non-irritating. Its effects on the parasites is perhaps very slight. While improving the patient's health, it does not remove the cause, though it has apparently some effect upon the pyrexia. In order that it may do any good it must be given in very large doses (grs. xv) by injection every seven or ten days, continued for several months.

4. Spleen puncture—It is practically safe if one uses a hypodermic needle and does not draw more than one or two drops of blood.*

Spleen puncture is not so safe as the author has stated.—*Ed.*

[Reprinted from the British Medical Journal, Vol. II, September 26, 1908]

# FATTY LIVER IN KALA-AZAR

In the British Medical Journal, May 30, 1908, I pointed out that in cases of kala-azar, the liver is frequently enlarged. This enlargement is in some cases due to an intracellular cirrhosis, as pointed out by Major Rogers.[1] In one case I observed extensive fatty changes in the liver, which, I consider, is a very rare condition in kala-azar. It has not, as far as I am aware, been described by any previous observer.

Condition on admission—The patient, æt. 18, was admitted into my wards with history of fever coming on irregularly for about eight months. He was markedly emaciated and cachectic. The spleen was slightly enlarged, extending about an inch below the costal arch. The liver extended about an inch and a half below the costal margin. The spleen blood showed a large number of Leishman-Donovan bodies. The lungs and the heart showed nothing abnormal. There was no complication at the time of admission into the hospital.

After history—After a fortnight he began to suffer from very obstinate diarrhœa, which terminated fatally. There was no ascites, and there were no enlarged veins in front of the abdomen nor other signs of portal obstruction. There was no œdema in the lower extremities. The fever was irregular, sometimes presenting the double remittent type and sometimes hectic. The patient died in the hospital twenty-five days after admission. He was treated with injections of atoxyl. A few days before death he suffered from an abscess in the right arm and also an inflammatory swelling in the left.

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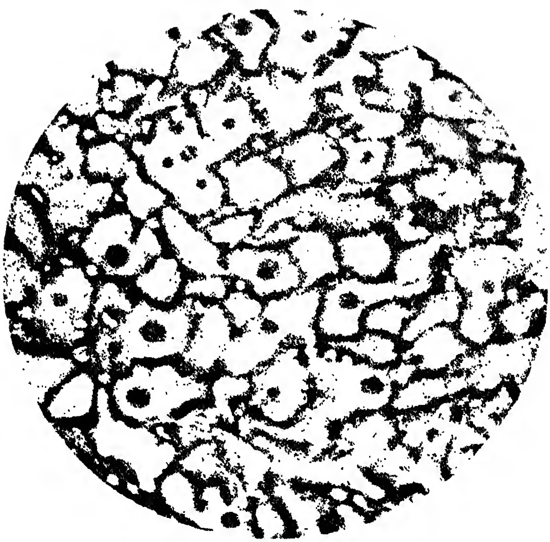


Section of liver showing extensive fatty degeneration of the cells, more about the peripheral portions of the lobules of the liver. (*Vide* para. 3).

Blood Count—Red corpuscles, 1,957,000 ; white, 2,723 ; proportion, 1 in 720.

*Post-mortem* Examination—The body was emaciated, subcutaneous fat diminished ; heart valves healthy ; lungs, nothing abnormal.

The liver was enlarged, smooth, and yellowish in colour. Its margin was somewhat rounded; on section it had a yellow mottled appearance. When the surface was scraped, droplets of fat could be seen on the knife. Microscopically (see Diagram) extensive fatty degeneration of the liver cells was noticed, rather more about the peripheral portions of the lobules of the liver. In many places the liver cells were shrivelled, and many contained small oil globules scattered throughout their protoplasm. In a few of the liver cells there could be seen deposits of granules of golden-brown pigment resembling hæmosiderin. In some of the lobules there was a very marked engorgement of blood in the centre (not shown in the Diagram). In some places there was a slight formation of new bile ducts. There was also a growth of delicate connective tissue appearing between the liver cells.

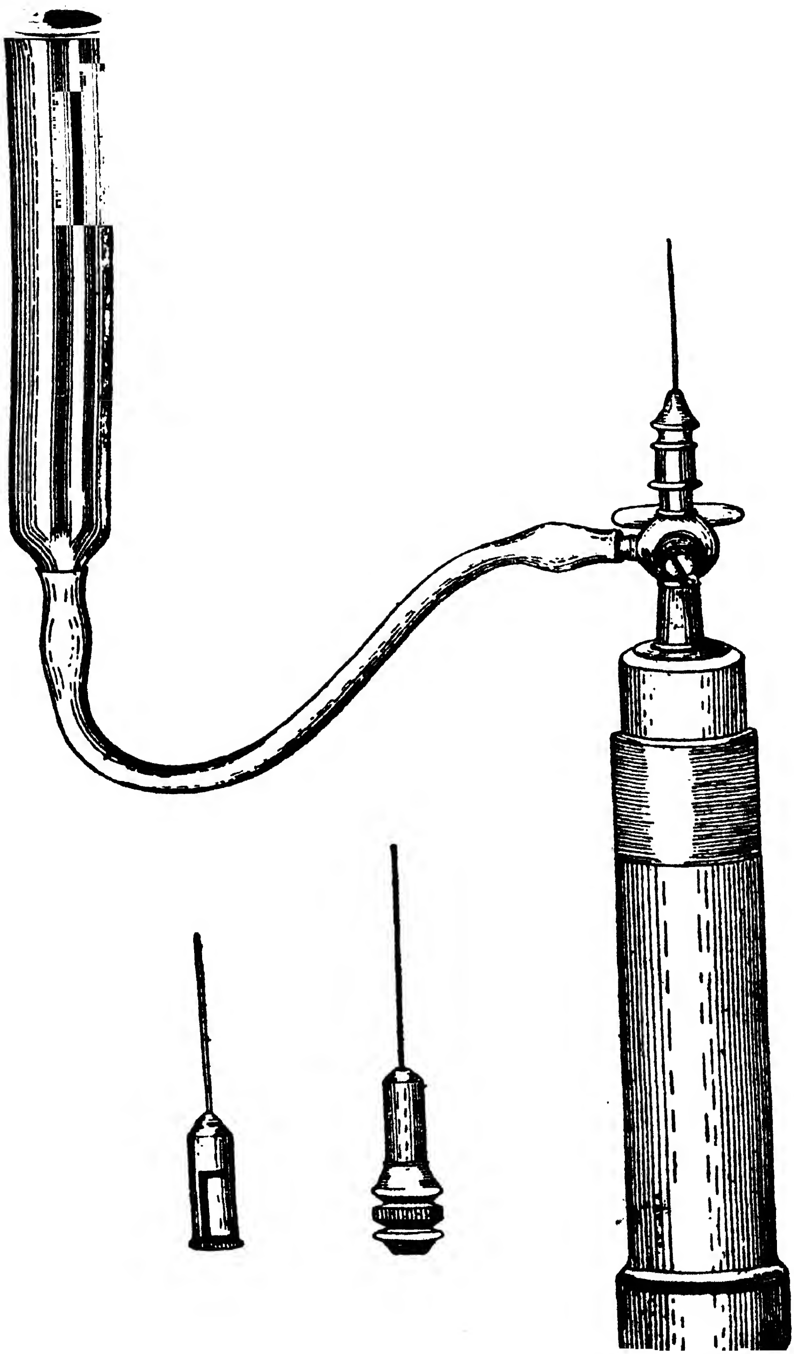
### *Reference*

1. L. Rogers (1908). *Fevers in the Tropics.*

[*Reprinted from the Indian Medical Gazette, Vol. L, No. 12, December, 1915*]

# A PRELIMINARY REPORT ON THE TREATMENT OF KALA-AZAR WITH INTRAVENOUS INJECTION OF METALLIC ANTIMONY

The use of metallic antimony in a state of finest subdivision in the treatment of kala-azar has not been noted by any previous observer. In a disease like kala-azar in which the parasites reside in such organs as the spleen and the bone marrow and in which very few parasites are found in the peripheral circulation, the use of the soluble salts of antimony may be followed by such quick elimination of the drug that they may be excreted before exerting any marked influence on the parasites in the spleen and the bone marrow, while metallic antimony may be retained for a much longer time if introduced into the circulation. In the treatment of the allied disease of trypanosomiasis by intravenous injection of tartar emetic or other soluble salts of antimony, it has been found that though they quickly free the peripheral blood from typanosomes, still the parasites remain in the internal organs. So far, therefore, the treatment of trypanosomiasis by means of intravenous injection of soluble salts of antimony has not been a permanent success. In kala-azar, in which the parasites live mostly in the internal organs, the same line of treatment may, from these theoretical considerations, be not expected to do much good. However, the treatment of kala-azar with tartar emetic and other soluble salts of antimony will form the subject of a future communication, though it may be remarked here, in passing, that good results with tartar emetic



have been recently reported by several observers. The present paper is a preliminary report, but the results obtained so far certainly justify further investigations.

The method of administration is the author's own and is described as follows :—

An all-glass 10 c.c. syringe is fitted with a three-way stop-cock, the remaining two ends of which are fitted to a platinum needle and a rubber tubing attached to the nozzle of a piece of glass tube respectively. A stout hypodermic needle with a specially constructed blunt canula inside may be used in place of an ordinary one. This canula when pushed through the needle will prevent the puncture of the vein a second time during the process of injection of the metallic antimony (see Figure). One grain of metallic antimony is made into a thoroughly homogeneous paste with sufficient liquid glucose in a glass mortar and then mixed with 20 c.c. normal saline. (The glucose added is just sufficient to make a 5 per cent solution with 20 c.c. of normal saline.)

The stop-cock is so arranged that the syringe may be made to communicate alternately with the needle or the glass tube by turning the stop-cock. Half the suspension is now sucked into the glass syringe, and after being freed from any bubbles of air, it is injected into a vein of the fore-arm. The glass tube is now filled with a portion of the remaining suspension, which is sucked into the glass syringe after freeing the rubber tubing of any air bubbles, and then the suspension is again injected. In this way the whole of the suspension is injected into the vein. Any sediment of antimony left inside the syringe is subsequently mixed with normal saline containing 5 per cent glucose, and then injected into the vein. This process is repeated several times till no antimony is left inside the syringe. About 40 to 45 c.c. of normal saline is required to inject 1 to $1\frac{1}{2}$ grs. of metallic antimony. In all the cases

recorded below, the diagnosis was made by the presence of Leishman-Donovan bodies in the splenic blood.

The first two cases were at first treated with tartar emetic and Plimmer's salt (sodium antimonyl tartrate). Then after some days they were treated with intravenous injection of metallic antimony.

Case No. 4 was treated with intravenous injection of metallic antimony and electargol. This patient had marked jaundice with œdema of the extremities, and there was a large amount of albumen in the urine. These completely disappeared during treatment.

Case No. 5 had *cancrum oris* on admission which disappeared after treatment.

The results so far obtained in these cases are recorded in the accompanying tables and the effects on the temperature are shown in the accompanying Charts.

It will be seen from the above that so far the results are encouraging and justify further investigation. No untoward results, such as plugging of the capillaries, have followed the intravenous injection of metallic antimony. Sometimes the patient suffered from rather severe diarrhœa, which, however, stopped in 24 to 48 hours.

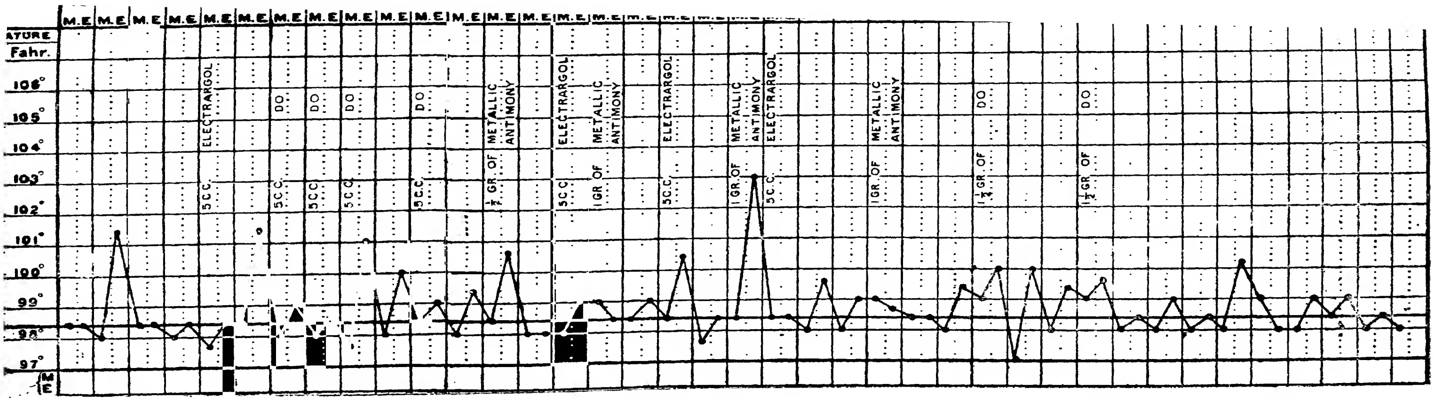
The advantages of intravenous injection of metallic antimony given in the above way are as follows :—

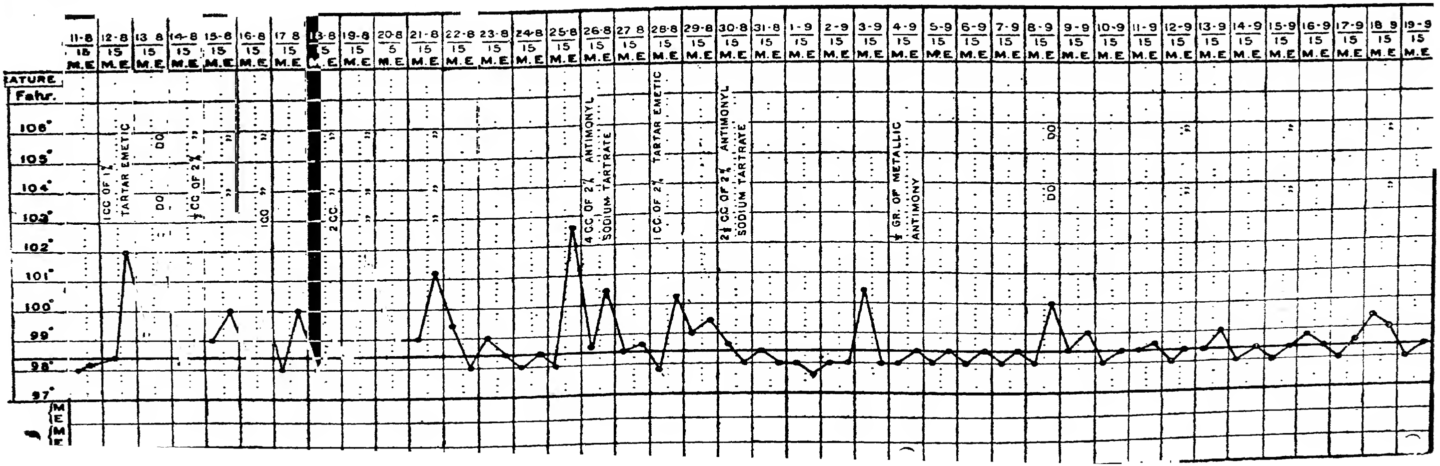
(1) A small quantity of normal saline is required for injecting the antimony. This is an advantage, as kala-azar patients are frequently liable to suffer from high fever and rigors after injection of large quantities of normal saline.

(2) An ordinary hypodermic needle may be used for the purpose, if it fits the stop-cock.

(3) The glucose is added to raise the specific gravity of the suspending fluid, and thus it reduces the settling down of the metallic antimony inside the injecting syringe to a minimum.

Case No. 6 was treated with electargol and tartar emetic and is recorded here for comparison.


ATURE
Fahr.
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
5 C.C. ELECTRARGOL
5 C.C. DO
5 C.C. DO
5 C.C. DO
5 C.C. DO
½ GR. OF METALLIC ANTIMONY
5 C.C. ELECTRARGOL
1 GR. OF METALLIC ANTIMONY
5 C.C. ELECTRARGOL
1 GR. OF METALLIC ANTIMONY
5 C.C. ELECTRARGOL
1 GR. OF METALLIC ANTIMONY
1½ GR. OF DO
1½ GR. OF DO


ATURE
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11-8 12-8 13-8 14-8 15-8 16-8 17-8 18-8 19-8 20-8 21-8 22-8 23-8 24-8 25-8 26-8 27-8 28-8 29-8 30-8 31-8 1-9 2-9 3-9 4-9 5-9 6-9 7-9 8-9 9-9 10-9 11-9 12-9 13-9 14-9 15-9 16-9 17-9 18-9 19-9
1 CC OF 1% TARTAR EMETIC
DO DO
½ CC OF 2%
1 CC
2 CC
4 CC OF 2% ANTIMONYL SODIUM TARTRATE
1 CC OF 2% TARTAR EMETIC
2½ CC OF 2% ANTIMONYL SODIUM TARTRATE
¼ GR. OF METALLIC ANTIMONY
DO DO

## TABLES SHOWING THE EFFECTS OF TREATMENT

### *Case I—Sitaram*

| Date. | Body weight. | Size of Spleen. | | Blood examination. |
|---|---|---|---|---|
| | | Lower border extends along the left nipple line | Longest transverse diameter begins from | |
| 3-8-15 | 3 st. 5 lbs. | 5″ below costal margin. | ¾″ left of middle line | R.B.C.—2,000,000<br>W.B.C.—2,400 |
| 19-8-15 | 3 st. 3½ lbs. | Do | Do | R.B.C.—2,400,000<br>W.B.C.—2,400<br>Hb.—38% |
| 27-8-15 | Do | Do | Do | R.B.C.—2,400,000<br>W.B.C.—3,000<br>Hb.—38% |
| 6-9-15 | Do | 4½″ Do | 2″ Do | R.B.C.—2,600,000<br>W.B.C.—6,400<br>Hb.—44% |
| 15-9-15 | Do | Do | Do | R.B.C.—3,600,000<br>W.B.C.—7,200<br>Hb.—56% |
| 27-9-15 | Do | 3″ Do | 2½″ Do | R.B.C.—4,000,000<br>W.B.C.—8,400<br>Hb.—60% |

### *Case II—Ramjitan*

| Date. | Body weight. | Size of Spleen. | | Blood examination. |
|---|---|---|---|---|
| | | Lower border extends along the left nipple line | Longest transverse diameter begins from | |
| 14-8-15 | 3st. 4lbs. | 4½″ below costal arch. | 1″ right of middle line. | R.B.C. 2,800,000<br>W.B.C.—5,600<br>Hb.—44% |
| 20-8-15 | 3st. 6 lbs. | Do | Do. | R.B.C.—2,600,000<br>W.B.C.—5,600<br>Hb.—46% |
| 3-9-15 | Do | Do | ¼″ left of middle line. | R.B.C.—2,400,000<br>W.B.C.—4,800<br>Hb.—48% |
| 18-9-15 | 3st. 8lbs. | Do | 3″ left of middle line. | R.B.C.—3,200,000<br>W.B.C.—8,800<br>Hb.—58% |

## *Case III—Mati*

| Date. | Body weight. | Size of Spleen. | | Blood examination. |
|---|---|---|---|---|
| | | Lower border extends along the left nipple line | Longest transverse diameter begins from | |
| 13-9-15 | ... | 5″ below costal arch. | 2″ right of middle line. | R.B.C.—2,000,000<br>W.B.C.—2,400<br>Hb.—46% |
| 27-9-15 | ... | 3″ Do | 1″ left of middle line | R.B.C.—2,800,000<br>W.B.C.—3,000 |
| 1-10-15 | .. | 2″ Do | Do | R.B.C.—2,800,000<br>W.B.C.—2,800<br>Hb.—52% |

## *Case IV—Durgadhan*

| Date. | Body weight. | Size of Spleen. | | Blood examination. |
|---|---|---|---|---|
| | | Lower border extends along the left nipple line | Longest transverse diameter begins from | |
| 21-8-15 | 5 st. 2 lbs. | 5½″ below costal arch. | 2½″ right of middle line. | R.B.C.—3,400,000<br>W.B.C.—4,000<br>Hb.—48% |
| 3-9-15 | 5 st. 3 lbs. | Do | Do | R.B.C—3,800,000<br>W.B.C.—3,200<br>Hb.—48% |
| 11-9-13 | 5 st. 6 lbs. | Do | Do | R.B.C.—4,000,000<br>W.B.C.—3,200<br>Hb.—62% |
| 27-9-15 | Do | 4″ Do | ½″ Do | R.B.C.—4,200,000<br>W.B.C.—6,000<br>Hb.—6[illegible]% |

## *Case V—Kasi*

| Date. | Body weight. | SIZE OF SPLEEN. | Blood examination. |
|---|---|---|---|
| 18-9-15 | ... | Spleen felt just below the costal arch. | R.B.C.—2,400,000<br>W.B.C.—4,800<br>Hb.-50% |
| 27-9-15 | ... | Spleen cannot be felt below the costal arch. | R.B.C.—2,800,000<br>W.B.C.—5,600<br>Hb.—52% |

## *Case VI—Narayan*

| Date. | Body weight. | SIZE OF SPLEEN. | | Blood examination. |
|---|---|---|---|---|
| | | Lower border extends along the left nipple line | Longest transverse diameter begins from | |
| 4-9-15 | 4 st. 6 lbs. | 5″ below costal arch. | ½″ left of middle line. | R.B.C.—2,800,000<br>W.B.C.—1,600<br>Hb.—46% |
| 11-9-15 | ... | 4½″ Do | 1¼″ Do | R.B.C.—2,600,000<br>W.B.C.—4,400<br>Hb.—50% |
| 18-9-15 | 4 st. 4½ lbs. | 4″ Do | 2″ Do | R.B.C.—2,600,000<br>W.B.C.—4,000<br>Hb.—54% |
| 2-10-15 | Do | Do | Do | R.B.C.—3,800,000<br>W.B.C.—6,400<br>Hb.—70% |

[Reprinted from the *Indian Medical Gazette*, Vol. LI, January, 1916]

# FURTHER OBSERVATIONS ON THE TREATMENT OF KALA-AZAR AND CASES TREATED WITH METALLIC ANTIMONY, SODIUM ANTIMONYL TARTRATE, FORM-ALDEHYDE AND OTHER DRUGS

## SECOND REPORT

This paper is a continuation of my previous paper which was published in the *Indian Medical Gazette*, December, 1915. It includes fresh cases treated with metallic antimony and other drugs and further observations about cases already reported.

*I. Cases treated with metallic antimony.*—

(*a*) Cases already reported :—

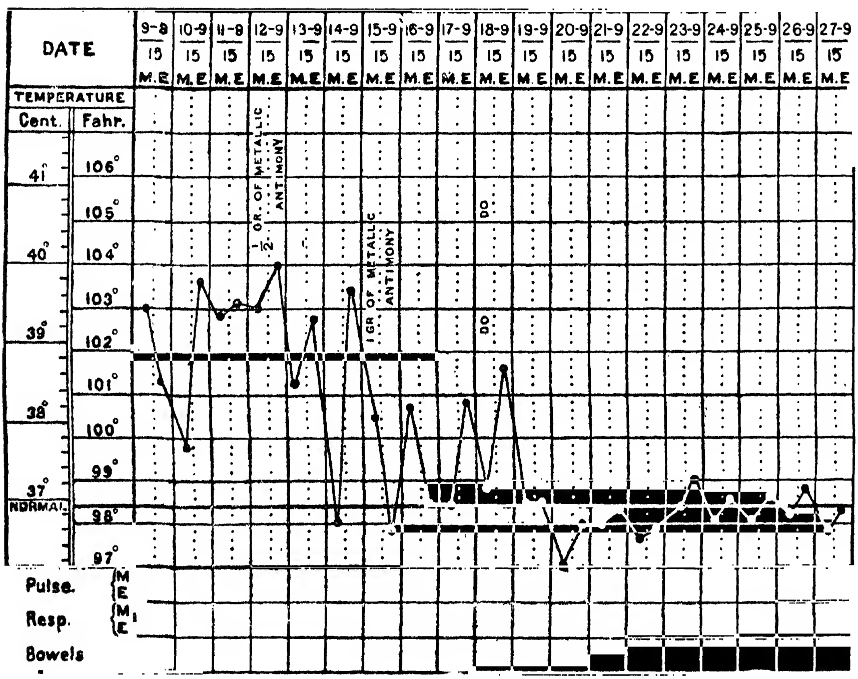
1. S.—The patient left hospital after being free from fever for nearly a month. He had altogether five injections of metallic antimony, half a grain each time.

Result of blood examination :—

R.B.C—2,000,000. W.B.C.—2,400 on 3rd August, 1915.

R.B.C.—4,000,000. W.B.C.—8,400. Hb.—60% on 27th September, 1915.

Size of spleen :—(1) 5″ below the costal margin on 3rd August, 1915. (2) 3″ below the costal margin on 15th September, 1915.




Temperature chart of a kala-azar case treated with three injections of metallic antimony half a grain each time. (Para. 1, No. 2).

2. R.—The patient left hospital after being free from fever for nearly a month. He had altogether three injections of metallic antimony, half a grain each time.

Result of blood examination :—

R.B.C.—2,800,000. W.B.C.—5,600. Hb.—44% on 4th August, 1915.

R.B.C.—3,800,000. W.B.C.—8,800. Hb.—66% on 29th September, 1915.

Size of spleen :—(1) 4½" below the costal margin on 14th August, 1915. (2) 3" below the costal margin on 3rd October, 1915. (3) Longest transverse diameter diminished by nearly 4".

3. M.—The patient left hospital after being free from fever for nearly three weeks. He had altogether four injections of metallic antimony.

First dose half a grain, and subsequent doses one grain.

Result of blood examination :—

R.B.C.—2,000,000. W.B.C.—2,400. Hb.—46% on 13th September, 1915.

R.B.C.—3,600,000. W.B.C.—4,400. Hb.—58% on 9th October, 1915.

Size of spleen :—(1) 5" below the costal margin on 13th September, 1915. (2) 1½" below the costal margin on 11th October, 1915.

4. D.—The patient left hospital after being free from fever for nearly a month and a half. He had altogether five injections of metallic antimony, first dose half a grain, second, third and fourth doses one grain each, fifth dose one and a half grains.

Result of blood examination :—

R.B.C.—3,400,000, W.B.C.—4,000. Hb.—48% on 21st August, 1915.

R.B.C.—4,600,000. W.B.C.—10,400. Hb.—68% on 29th October, 1915.

Size of spleen :—(1) $5\frac{1}{2}''$ below the costal margin on 21st August, 1915. (2) Cannot be felt below the costal margin on 3rd November, 1915.

5. K.—The patient is still in hospital. He had a very severe attack of acute enteritis and also suffered from pleurisy for a few days. For some time his condition was very serious. As mentioned in my previous paper he had *cancrum oris* at the time of admission. He has been free from fever for nearly a month and a half, except for a short period when he had pleurisy. He had altogether two injections of metallic antimony, first dose, half a grain and second dose, one grain.

Result of blood examination :—

R.B.C.—2,400,000. W.B C.—4,800. Hb.—50% on 18th September, 1915.

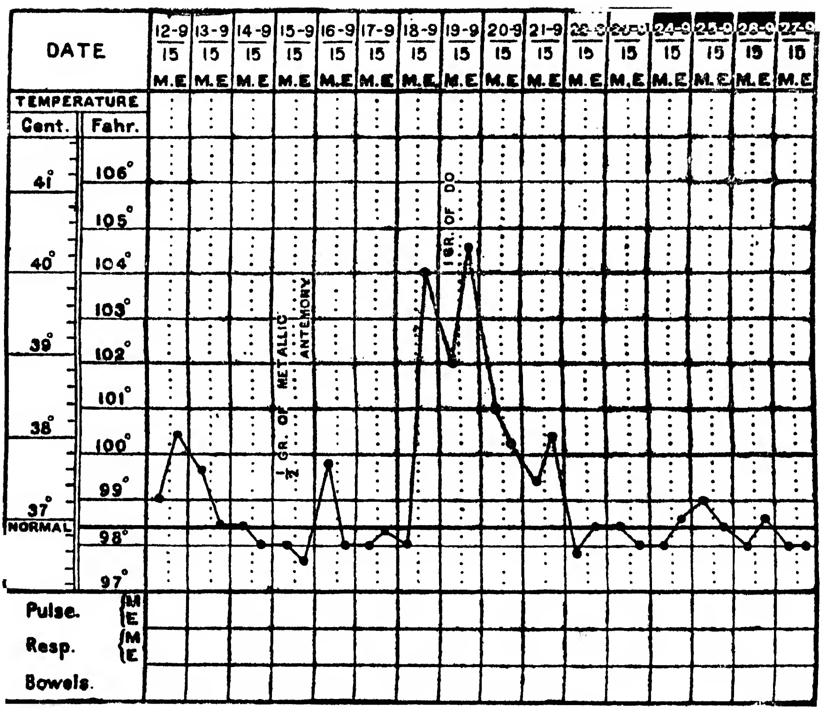
R.B.C.—2,600,000. W.B.C.—5,000. Hb.—55% on 28th November, 1915.

Size of spleen :—(1) Just felt below the costal margin on 18th September, 1915. (2) Cannot be felt below the costal margin on 28th November, 1915.

The general condition is improved and the patient is having a slow convalescence. The *cancrum oris* is cured but the patient in still very feeble.

(*b*) New cases :—

Three more cases were treated with metallic antimony which are recorded here.




Temperature chart of a kala-azar case treated with two injections of metallic antimony. (Case No. 5. K).

6. L.—Patient was suffering from high fever for some time before admission. He had altogether five injections, first dose being half a grain ; second, third, and fourth doses one grain each and fifth dose one and a half grains. (See Table I and temperature Chart I.) The patient is doing very well.

Size of spleen :—(1) 4″ below the costal margin on 12th October, 1915. (2) Cannot be felt below the costal margin on 9th November, 1915.

7. Putu—The patient had been in hospital for a short time and has left since.

8. Abdul—The patient had been in hospital for a short time and his case will be reported later on.

*II. Treatment with intravenous injection of galyl.*—

The brilliant results obtained with galyl in the treatment of sleeping sickness led me to try this drug in four cases. Each had one injection of ·25 gramme but no good results have followed its use. The drug should, however, be given a more extended trial, which is impossible in a hospital on account of its cost.

*III. Treatment with intravenous injection of formaldehyde.*—

The patient, named Gopi, a hospital servant, was admitted into my wards on 11th June, 1915, suffering from fever of remittent type. He had another attack a short time ago. On admission his spleen was found to extend four inches below the costal margin, and on spleen puncture a large number of Leishman-Donovan bodies were found. The patient was treated with intravenous injection of formaldehyde. Three injections of 40 c.c. of a solution of formaldehyde in normal saline (1 in 4,000) were given. (See temp. Chart II.)

The patient left hospital when his spleen extended two inches below the costal margin, having much improved in health. He went to his native village and has come back in

perfectly healthy condition. This is one of the few cases in hospital which has been under my observation for nearly six months. His general condition is now very much improved and he is doing his work in hospital as a perfectly healthy man. (See Table II for his blood condition.)

*IV. Treatment with Plimmer's salt (sodium antimonyl tartrate).*—I have already reported two cases treated with this salt which were subsequently treated with metallic antimony. Four more cases have been treated with the same drug.

The following are the notes on one of them :—

The patient, M., came under my treatment on 1st October, with enlarged spleen extending about 4 inches below the costal arch. He was at first treated with galyl which did him no good as the temperature chart and the result of the blood examination will show. After 10 days he was put on a course of Plimmer's salt. The results of blood examination and the temperature Chart are appended herewith. (Chart III and Table III.)

The results seem to be, so far, similar to what has been reported in cases treated with tartar emetic. The doses are similar to what I generally follow in the case of tartar emetic. The patient seems to have much improved in health. The salt used by me was perfectly pure, having been specially prepared for my work. I have given Rai H. N. Ghose Bahadur some of this pure salt to use in his wards.

*V. Treatment with intravenous injection of tartar emetic and sodium antimonyl tartrate.*—Three cases were treated with both the drugs, the idea being that when the higher doses were used, the sodium salt was preferable. One of these patients, a girl named K., æt. 11, has remarkably improved under this treatment. (See Chart IV and Table IV.)

Another case, a boy, æt. 11, was given from ½ c.c. to 4 c.c. of 2 per cent solution of tartar emetic for 7 days and then he was treated with 5 c.c. to 6 c.c. of 2 per cent solution

of the sodium salt every two days for some time and afterwards every four days. He was altogether given 14 injections. The result has been that the spleen has gone down from 4″ to ½″ below the costal margin and the temperature has come down to normal. The R.B.C., W.B.C., and Hb. have gone up from 4,400,000, 2,910, and 35% before treatment to 5,600,000, 9,100, and 55% respectively after treatment in a month's time.

The highest dose of the sodium salt used for an adult was 7 c.c. of 2 per cent solution.

*VI. Treatment with tartar emetic and other drugs combined.*—In one case the patient was put on berberine sulph. (2½ grs.) and nuclein capsules thrice a day for two months and a half along with tartar emetic given intravenously. The result, so far obtained, does not seem to be different from what has been obtained from tartar emetic alone. In another case berberine sulph. has been used intravenously for some time. In one case galyl was administered after a course of treatment with tartar emetic but this did not influence the course of the disease.

*Retention of antimony in the tissues when introduced into the system as metallic antimony.*—In my last paper I pointed out the possibility of the retention of antimony in the tissues, such as the spleen, for a much longer time when introduced into the circulation as metallic antimony than when introduced in the form of soluble salts. The following investigations have confirmed this view:—A patient treated with tartar emetic died of dysentery 24 hours after the last injection; no trace of antimony could be detected in the spleen and the liver by chemical examination. Another patient who had an injection of half a grain of metallic antimony also died of dysentery 132 hours after the injection, and traces of antimony could still be detected in the spleen and the liver by chemical examination, thus showing that antimony introduced into the circulation as

metallic antimony is retained in the spleen and the liver for a much longer time than when introduced as a soluble salt.

I am indebted to Rai Chunilal Bose Bahadur, Chemical Examiner to the Government of Bengal, for the above chemical analyses.

## CONCLUSIONS

(1) So far as the above cases go to prove, metallic antimony seems to produce very marked beneficial effects in kala-azar, and the effects tend to be permanent.

(2) The soluble salts of antimony, such as tartar emetic and sodium antimonyl tartrate, are also very beneficial in the treatment of the disease.

(3) Metallic antimony introduced into the circulation remains in the spleen and the liver for a much longer period than when introduced in the form of soluble salts. It may, therefore, be expected to give rise to more marked and permanent results. Not more than five injections have been given to any of my cases. It appears that not more than three or four injections are required to bring about what appears to me a permanent cure.

(4) The results obtained from combining antimony treatment with other drugs such as galyl, berberine sulph. and nuclein, do not, so far, seem to differ from what follows the treatment with tartar emetic itself.

(5) Galyl has been tried in four cases without any effect, but evidently it must be given a further trial before its effect can be determined.

(6) One remarkable case of recovery has been recorded following intravenous injection of formaldehyde.

(7) Attempts are being made to prepare a colloidal solution of the metallic antimony, and if this succeeds, the colloidal solution of the metal will perhaps be the ideal preparation of antimony to be adopted in the treatment of kala-azar.

A portion of this paper was read before the Calcutta Medical Club last November, and some of the cases were mentioned in the meeting of the Medical Section of the Asiatic Society of Bengal last December.

### TABLE I

L.—*Patient treated with metallic antimony*

| Date. | Size of spleen. | Result of blood examination |
|---|---|---|
| 13-10-15 | 4″ below the costal arch | R.B.C.—2,140,000<br>W.B.C.—2,600<br>Hb.—32% |
| 23-10-15 | 1″ do. | R.B.C.—2,500,000<br>W.B.C.—2,100 |
| 1-11-15 | Cannot be felt below the costal arch. | R.B C – 2,200,000<br>W.B C.—4,000<br>Hb.—32% |

### TABLE II

Gopi—*Patient treated with formaldehyde*

| Date. | Size of spleen. | Result of blood examination. |
|---|---|---|
| 12-6-15 | 4″ below the costal margin | R.B.C —3,800,000<br>W.B.C.—3,200 |
| 5-7-15 | 2″ do. | R.B.C.—4,200,000<br>W.B C.– 4,400<br>Hb.—54% |
| 1-11-15 | Cannot be felt below the costal margin. | R.B C.—5,000,000<br>W.B.C.– 11,200<br>Hb.—85% |

## Table III

M.—*Patient treated with Plimmer's salt (sodium antimonyl tartrate)*

| Date. | Size of spleen. | Result of blood examination. |
|---|---|---|
| 7-10-15 | 4″ below the costal arch | R.B.C.–2,400,000<br>W.B.C.—1,200<br>Hb.–38% |
| 13-10-15 | 4″ do. | R B.C.– 1,800,000<br>W.B.C.—2,000<br>Hb.—30% |
| 27-10-15 | 2″ do | R.B.C.—2,600,000<br>W.B.C.—3,600<br>Hb.--40% |
| 9-11-15 | 1″ do. | R.B.C.—3,200,000<br>W.B.C.—6,000<br>Hb.—55% |

## Table IV

*Patient treated with tartar emetic and sodium antimonyl tartrate*

| Date. | Body weight. | Size of spleen. | Result of blood examination. |
|---|---|---|---|
| 26-10-15 | 2 st. 10 lbs. | 4″ below the costal arch. | ... R.B.C.—2,950,000<br>W.B.C.—3,200 |
| 20-11-15 | ... | 3″ do. | R.B.C.—2,800,000<br>W.B.C.—2,400<br>Hb.—38% |
| 21-11-15 | ... | 2″ do. | R.B.C.—2,900,000<br>W.B.C.—5,200<br>Hb.—42% |
| 3-12-15 | 3 st. 2 lbs. | Cannot be felt below the costal arch. | R.B.C.—3,000,000<br>W.B.C.– 5,600<br>Hb.—44% |
| 15-12-15 | ... | do. | R.B.C.—3,900,000<br>W.B.C.—10,600<br>Hb.—60% |

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# THIRD REPORT ON THE TREATMENT OF KALA-AZAR WITH SPECIAL REFERENCE TO THE USE OF ANTIMONY AND FORMALDEHYDE *

This paper is a continuation of my papers on the treatment of kala-azar published in the *Indian Medical Gazette* last December and January. It includes fresh cases and further observations about cases already reported.

The paper can be conveniently divided into the following parts :—

1. Cases treated with metallic antimony.
2. Cases treated with compounds of antimony.
3. Cases treated with intravenous injections of antiseptics, e.g., formaldehyde, eusol.
4. Alkaloidal therapy.

## I. Cases treated with Metallic Antimony

### (*a*) *Colloidal Metallic Antimony*

In my last communication in the *Indian Medical Gazette* I pointed out that colloidal metallic antimony would perhaps be the ideal preparation of antimony to be used in the treatment of kala-azar.

The use of colloidal metallic antimony in the treatment of kala-azar has not been noted by any previous observer. Reference to its use in therapeutics is very meagre. The following cases are therefore of much interest as being the

* Read before the Medical Section of the Asiatic Society of Bengal, on 26th April, 1916.

first recorded cases showing marked benefit by the use of colloidal antimony.

The patient Bhuban was admitted into my wards on 16th February, 1916. He was cachectic and much emaciated at the time of admission. The spleen extended 3″ below the costal arch and there was a large number of L. D. bodies present in the splenic blood. He was at first treated with intramuscular and subsequently with intravenous injections of colloidal metallic antimony. No unpleasant symptoms followed the intravenous injections. It was found that very quickly satisfactory results followed the treatment as will be seen from the following :—

RESULT OF BLOOD EXAMINATION

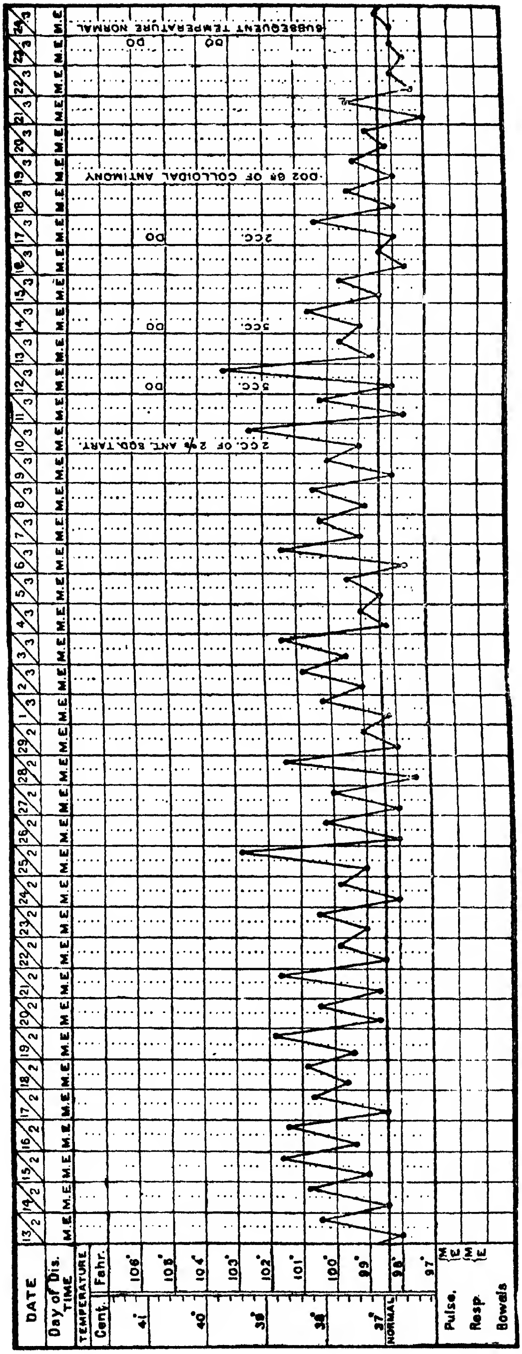
| | | | | |
|---|---|---|---|---|
| R. B. C.—2,600,000 | W. B. C.—4,800 | Hb.—40% | on | 17-2-16 |
| R. B. C.—4,100,000 | W. B. C.—5,600 | Hb.—50% | on | 24-2-16 |
| R. B. C.—4,000,000 | W. B. C.—6,600 | Hb.—54% | on | 6-3-16 |
| R. B. C.—4,200,000 | W. B. C.—7,800 | Hb.—54% | on | 14-3-16 |
| R. B. C.—4,300,000 | W. B. C.—9,200 | Hb.—70% | on | 30-3-16 |

Size of spleen—3″ below the costal margin in the left nipple line on 20-2-16 and 1″ on 14-3-16.

Doses of colloidal antimony given :—

Four intramuscular injections of ·001 grm. on successive days and 19 intravenous injections of ·002 grm. on the next successive days. The 20th injection was given eight days after the 19th injection.

The second case treated with colloidal antimony was Amiya. She was at first treated with intravenous injections of berberine sulphate and subsequently with sodium antimonyl tartrate. As will be seen below, she got no benefit from berberine. The administration of sodium antimonyl tartrate was followed by severe diarrhœa. I, therefore, decided




Temperature chart of a kala-azar case, treated with antimony sodium tartrate after berberine failed (*Vide* Chart (a), p. 303, para. 2), subsequently cured by colloidal metallic antimony. Patient.—Amiyo.—last para., Chart (b).

to put her on colloidal antimony and, as will be seen below, the results so far have been very satisfactory.

*Four Injections of Sodium Antimonyl Tartrate*

| | | | | |
|---|---|---|---|---|
| R. B. C.—3,800,000 | W. B. C.—3,200 | Hb.—40% | on | 7-3-16 |
| R. B. C.—4,100,000 | W. B. C.—2,600 | Hb.—40% | on | 15-3-16 |

*Treatment with Colloidal Antimony*

| | | | | |
|---|---|---|---|---|
| R. B. C.—4,300,000 | W. B. C.—3,800 | Hb.—44% | on | 24-3-16 |
| R. B. C.—4,300,000 | W. B. C.—7,400 | Hb.—48% | on | 14-4-16 |

She had altogether 15 injections of ·002 grm. and 5 injections of ·003 grm. of the colloid intravenously.

*Observations*

From the above it will be seen that the results so far seem to be very encouraging. On one occasion the patient Bhuban was given ·004 grm. of the colloid and this was followed by a sharp rise of temperature. The patient left hospital markedly improved in health and in body weight. On the day of discharge the spleen could not be felt below the costal arch. The second case is still in the hospital.

(*b*) *Cases treated with metallic antimony in a state of fine subdivision as an impalpable powder* :—

I have some more cases to report which were treated with this drug since the publication of my last paper. This would make a series of twelve cases which have been treated with metallic antimony alone or with metallic antimony after other drugs were tried.

(1) Abdul.—The patient has been free from fever for nearly four months. He had altogether seven injections of metallic antimony, once a week, starting with ½ gr. and ending with 1½ grs. [ In my last paper it was reported that

treatment with metallic antimony was just begun in this case.]

| | | | | |
|---|---|---|---|---|
| R. B. C.—3,200,000 | W. B. C.— 4,000 | ........... | on | 5-11-15 |
| R B. C.—3,600,000 | W. B. C.— 6,600 | Hb.—62% | on | 23-11-15 |
| R. B. C.—4,700,000 | W. B. C.—10,200 | Hb.—70% | on | 14-12-16 |
| R. B. C.—4,800,000 | W. B. C.—10,600 | Hb.—82% | on | 4-1-16 |
| R. B. C.—5,600,000 | W. B. C.— 9,600 | Hb.—76% | on | 16-2-16 |

Size of spleen—5″ below the costal margin on 21-10-15 and ½″ on 16-2-16.

(2) Abdul Aziz—was for some time treated with intravenous injections of narcotine. As the patient was not improving except in the leucocyte count and as the L. D. bodies were present even after ten injections of the drug, he was put on metallic antimony. He had altogether three injections of metallic antimony, each dose being one grain. (*Vide* Temperature Chart.)

RESULT OF BLOOD EXAMINATION

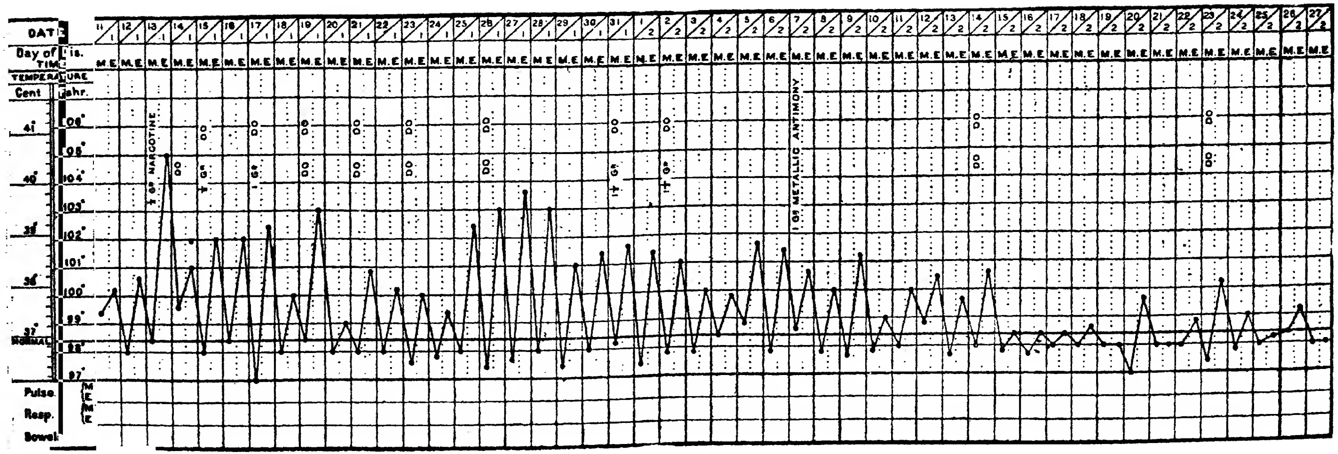
*Treatment with Narcotine*

| | | | | |
|---|---|---|---|---|
| R. B. C.—2,900,000 | W. B. C.—2,400 | Hb.—46% | on | 13-1-16 |
| R. B. C.—3,400,000 | W. B. C.—4,600 | Hb.—48% | on | 4-2-16 |

*Treatment with Metallic Antimony*

| | | | | |
|---|---|---|---|---|
| R. B. C.—3,600,000 | W. B. C.— 5,400 | Hb.—50% | on | 14-2-16 |
| R. B. C.—4,100,000 | W. B. C.— 8,200 | Hb.—56% | on | 23-2-16 |
| R. B. C.—4,500,000 | W. B. C.—10,400 | Hb.—60% | on | 4-4-16 |

(3) Patient—N.—He was at first treated with tartar emetic and his case was reported in the December number of the *Indian Medical Gazette*. He was discharged from hospital much improved on 5th October, 1915, and was readmitted on 8th November, 1915. He had altogether four injections of metallic antimony, first two doses being one grain and last two doses one grain and a half each.




Temperature chart of a kala-azar case, treated first with intravenous narcotine with no improvement in temperature—subsequently treated with metallic antimony (Case No. 2, patient—Abdul Aziz. Ref. p. 302).

(4) Ascrit.—He was at first treated with eusol. But as this was followed by slight improvement, he was given 3 injections of metallic antimony. As a result of this the spleen which at first extended 5″ below the costal arch could not be felt there.

RESULT OF BLOOD EXAMINATION

| | | | | |
|---|---|---|---|---|
| R. B. C.—4,200,000 | W. B. C.— 3,200 | Hb.—58% | on | 13-2-16 |
| R. B. C.—4,900,000 | W. B. C.— 7,800 | Hb.—66% | on | 10-4-16 |
| R. B. C.—4,900,000 | W. B. C.—12,400 | Hb.—66% | on | 18-4-16 |

(5) Saha.—He had four injections of metallic antimony. The blood count was : R. B. C.—2,600,000. W. B. C.—2,400, Hb.—35% before treatment and R. B. C.—4,500,000, W. B. C.—10,000, Hb.—70% after treatment.

*Observations*

It was seen that every one of the above cases treated with intravenous injections of metallic antimony markedly improved under the treatment, the improvement being noticed under the following heads: (1) improvement of blood condition ; (2) diminution in the size of spleen ; (3) subsidence of the fever and (4) absence of the L. D. bodies from the spleen. So far, therefore, these cases may be considered as cured. In the above series of cases I have not taken into account a case which died of chronic dysentery five days after he had one injection of metallic antimony.

## II. CASES TREATED WITH COMPOUNDS OF ANTIMONY

### A. *Cases treated with Soluble Salts of Antimony, Tartar Emetic and Sodium Antimonyl Tartrate*

Since my earlier communications on the treatment of kala-azar with antimony and its salts, I have treated some more cases with sodium antimonyl tartrate either alone or combined with tartar emetic.

It was observed that most of these cases improved under the treatment, the improvement being noticed under the same heads as described above (see under I, pp. 291-95). The total number of injections given to some of my cases are enumerated below :—

(1) M (already reported)—7 injections of sodium antimonyl tartrate (up to the last report) and 7 injections since.

(2) Nandy—12 injections of sodium antimonyl tartrate.

(3) Sing—20 injections of sodium antimonyl tartrate.

(4) Das (already reported)—7 injections of tartar emetic + 10 injections of sodium antimonyl tartrate.

(5) Kamala (already reported)—6 injections of tartar emetic + 8 injections of sodium antimonyl tartrate.

(6) Satya—6 injections of tartar emetic.

(7) Kar—11 injections of tartar emetic + 5 injections of sodium antimonyl tartrate.

(8) Saha—8 injections of sodium antimonyl tartrate (treatment discontinued and patient put on metallic antimony, as there was no improvement in the blood condition after 8 injections).

(9) Sishir—19 injections of sodium antimonyl tartrate (treatment still continued).

(10) Souren—9 injections of sodium antimonyl tartrate + 2 injections of tartar emetic (treatment still continued).

One of my youngest cases treated with sodium antimonyl tartrate was a patient aged 3 years. Up to now, the highest dose she has received is 2 c.c. of 2 per cent solution of the salt. She is now being treated with the sodium and the potassium salts alternately and is progressing favourably.

The injections were stopped after the patient had been free from fever for some time, the spleen had gone down to almost under the costal arch, the Leishman-Donovan bodies had disappeared from the splenic blood and the leucocyte count had been high.

The results of examination of blood in some of my cases have been shown in my previous papers. All the cases that have been already reported in the papers have since been free from fever and have markedly improved in health. The latest report of examination of blood of some of these cases treated with the soluble salts is as follows :—

(1) Patient M (reported in the January number of the *Indian Medical Gazette*), R. B. C.—4,500,000, W.B.C.—8,500, Hb.—75% on 15th February, 1916 (patient had altogether 14 injections of sodium antimonyl tartrate).

(2) Das (reported in the above paper), R. B. C.—4,500,000, W. B. C.—9,000, Hb.—75% on 16th February, 1916 (patient had 7 injections of tartar emetic and 10 of sodium antimonyl tartrate).

(3) Nandy—R. B. C.—2,800,000, W. B. C.—4,600, Hb.—38% on 20th December, 1915; R. B. C.—5,200,000, W. B. C.—8,500, Hb.—70% on 28th February, 1916 (patient had 12 injections of sodium antimonyl tartrate).

*Observations*

It requires to be seen whether any of the cases mentioned above would get a relapse after some months. If they do not, then they may be considered as cured. The doses and the intervals between successive injections were described in my previous papers. The alternate administration of the sodium and the potassium salts seems to be attended with the best results.

*Metallic Antimony and its Soluble Salts compared*

It is at present difficult to state from clinical experience whether metallic antimony or its soluble salts would constitute the best form of treatment in kala-azar, though, as stated in my previous papers, from theoretical grounds and from comparison with trypanosomiasis, metallic antimony holds

out the best chances of cure. The following facts have, however, at present, been observed :—

(1) The number of injections of metallic antimony required is much less than that of its soluble salts to produce the beneficial effects in kala-azar. (Three to four injections are generally required.)

(2) The effect is more quick after injections of metallic antimony.

(3) The toxic symptoms were less marked and the beneficial effects more permanent after injections of metallic antimony than after injections of its soluble salts in the following case—

The patient Narayan (already reported in the December issue of the *Indian Medical Gazette*) was at first treated with tartar emetic and was apparently cured after seven injections. He was discharged from the hospital on the 5th October, 1915, and was re-admitted on the 8th November, 1915. He still had an enlarged spleen, and though he was free from fever, his general condition seemed to have somewhat deteriorated. He was at first given an injection of galyl which apparently did him no good. Then he was given 3 c.c. of 2 per cent solution of sodium antimonyl tartrate. This was followed by severe enteritis and could not be continued. He was then put on a course of treatment with intravenous injections of metallic antimony, and this was quickly followed by remarkable improvement in the blood count and in general health. The following is a short account of his blood count after the various treatments :—

R. B. C.—2,800,000, W. B. C.—1,600, Hb.—46% on 4-9-15.

R. B. C.—3,800,000, W. B. C.—6,400, Hb.—70% on 2-10-15 after 7 injections of tartar emetic.

R. B. C.—3,600,000, W. B. C.—5,600 Hb.—62% on 11-11-15 (on re-admission into hospital).

R. B. C.—3,400,000, W. B. C.—4,400, Hb.—58% on 21-11-15 ('3 gramme galyl injected on 17-11-15).

R. B. C.—3,300,000, W. B. C.—6,400, Hb.—54% on 20-12-15 (3 c.c. of 2 per cent solution of sodium antimonyl tartrate injected on 27-11-15).

R. B. C.—3,800,000, W. B. C.—13,800, Hb.—68% on 17-1-16 (one grain of metallic antimony injected on 15-12-15 and one and a half grains on 26-12-15 and 4-1-16).

It will be seen in the above case that the greatest benefit followed the injection of metallic antimony.

The comparison of the effects of colloidal metallic antimony with those of metallic antimony in a state of fine subdivision as an impalpable powder is reserved for a future communication.

*Observations*

The following facts may be mentioned here :—

(1) Colloidal metallic antimony is administered in very small doses ('002 gramme).

(2) The colloid may be injected in 2 c.c. suspension and is therefore most convenient to use.

(3) No unpleasant symptoms follow the injection of colloidal antimony.

### B. *Case treated with Colloidal Solution of Sulphide of Antimony*

One case is being treated with colloidal sulphide of antimony, given subcutaneously in doses of '002 gramme.

## III. Treatment with Intravenous Injections of Antiseptics

(*a*) I have already reported an apparently cured case after treatment with intravenous injections of formaldehyde. Since the above case was published, two more cases have been treated with the same drug.

(1) The patient, named Kasi, is one of them and was reported before. As will be seen from my previous paper, though the patient got rid of his fever and his general condition improved after two injections of metallic antimony, his blood count was still below the normal. I decided to try intravenous injections of formaldehyde and a remarkable improvement in his blood followed, as will be shown below :—

| | | | |
|---|---|---|---|
| R. B. C.—2,600,000, | W. B. C.—5,000, | Hb.—55% | on 28-11-15 |
| R. B. C.—2,600,000, | W. B. C.—4,200, | Hb.—50% | on 3-12-15 |
| R. B. C.—3,700,000, | W. B. C.—9,800, | Hb.—62% | on 4-1-16 |

*Doses of Formaldehyde given*

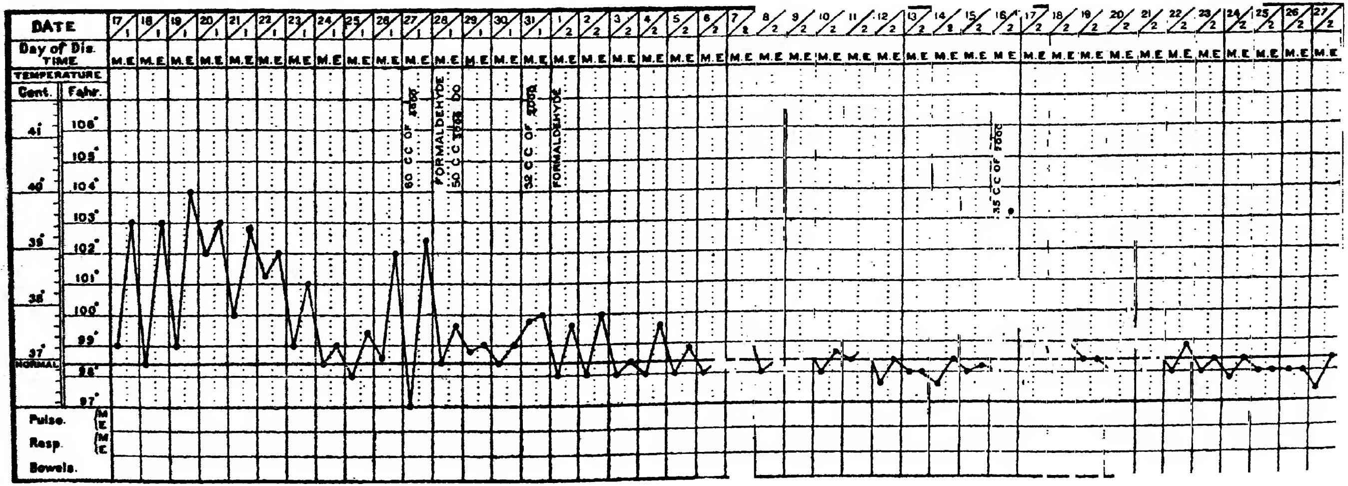
| | | | | | |
|---|---|---|---|---|---|
| 18 c.c. of $\frac{1}{4000}$ | solution | of formaldehyde | injected | on | 5-12-15 |
| 36 c.c. of do. | ,, | ,, | ,, | on | 6-12-15 |
| 35 c.c. of $\frac{1}{3000}$ | ,, | ,, | ,, | on | 7-12-15 |
| 36 c.c. of do. | ,, | ,, | ,, | on | 9-12-15 |
| 34 c.c. of $\frac{1}{2000}$ | ,, | ,, | ,, | on | 14-12-15 |

(2) The patient, named Tetari, was admitted on 17-1-16 into my wards. She was given three injections of formaldehyde and as a result of this there was a remarkable improvement in her condition. (*Vide* Temperature Chart.)

| | | | |
|---|---|---|---|
| R. B. C.—2,500,000, | W. B. C.— 3,000, | Hb.—42% | on 24-1-16 |
| R. B. C.—2,600,000, | W. B. C.— 3,800, | Hb.—42% | on 31-1-16 |
| R. B. C.—4,000,000, | W. B. C.—11,200, | Hb.—54% | on 7-2-16 |
| R. B. C.—3,900,000, | W. B. C.— 7,900, | Hb.—56% | on 15-2-16 |
| Size of spleen—4″ | below the costal | margin | on 24-1-16 |
| Size of spleen—½″ | below the costal | margin | on 20-2-16 |

*Doses of Formaldehyde given*

| | | | | | |
|---|---|---|---|---|---|
| 60 c.c. of $\frac{1}{4000}$ | sol. | of formaldehyde | injected | on | 27-1-16 |
| 50 c.c. of $\frac{1}{3000}$ | ,, | ,, | ,, | on | 28-1-16 |
| 32 c.c. of $\frac{1}{2000}$ | ,, | ,, | ,, | on | 31-1-16 |
| 35 c.c. of $\frac{1}{2000}$ | ,, | ,, | ,, | on | 16-2-16 |




Temperature chart of a kala-azar case, treated with three injections of formaldehyde with remarkable improvement. (Case No. 2, patient—Tetari).

(*b*) The next antiseptic used was eusol, prepared after Lorrain Smith's formula.

The patient, named Ascrit, was admitted into my wards on 17th January, 1916. The spleen extended 5″ below the costal margin in the parasternal line and a large number of L. D. bodies were found on spleen puncture. He was given altogether 15 injections of eusol. There was a slight improvement in the blood count, but the results were not so satisfactory as those following treatment with antimony, as will be seen from the following notes :—

R.B.C.—3,500,000, W.B.C.—3,800, Hb.—48% on 19-1-16
R.B.C.—3,900,C00, W.B.C.—4,200, Hb.—50% on 31-1-16
R.B.C.—4,200,000. W.B.C.—5,800, Hb.—58% on 19-2-16
R.B.C.—4,300,000, W.B.C.—4,800, Hb.—60% on 2-3-16
Size of spleen—not diminished.

*Doses of Eusol given*

The doses varied from 55 c.c. to 200 c.c. of eusol given intravenously on successive days.

As stated above the patient was subsequently treated with metallic antimony with brilliant results.

It will be seen that formaldehyde acted like a specific in the three cases in which it was used, the effect being noticed under the same heads as those described under antimony and its salts. Evidently, however, the drug must be given a more extended trial before its specific action is established beyond doubt. In the single case mentioned above in which eusol has been tried the results so far seem to be slightly promising but much less marked than with formaldehyde. It may be mentioned here that there were no untoward results following intravenous injection of eusol. The objections to formaldehyde, however, lie in the fact that it is liable to decomposition and different samples contain different strengths. It was therefore decided to use a preparation of formaldehyde which is more stable and obtainable in

crystalline form, and this is formaldehyde-sodium bisulphite. A case is being treated with this drug and will be reported later on.

## IV. ALKALOIDAL THERAPY

Attempts were made to discover an alkaloid which would exert a specific action in kala-azar.

In one case, quinine was given intravenously in the form of quinine bihydrochloride but no impression was made on the course of the disease.

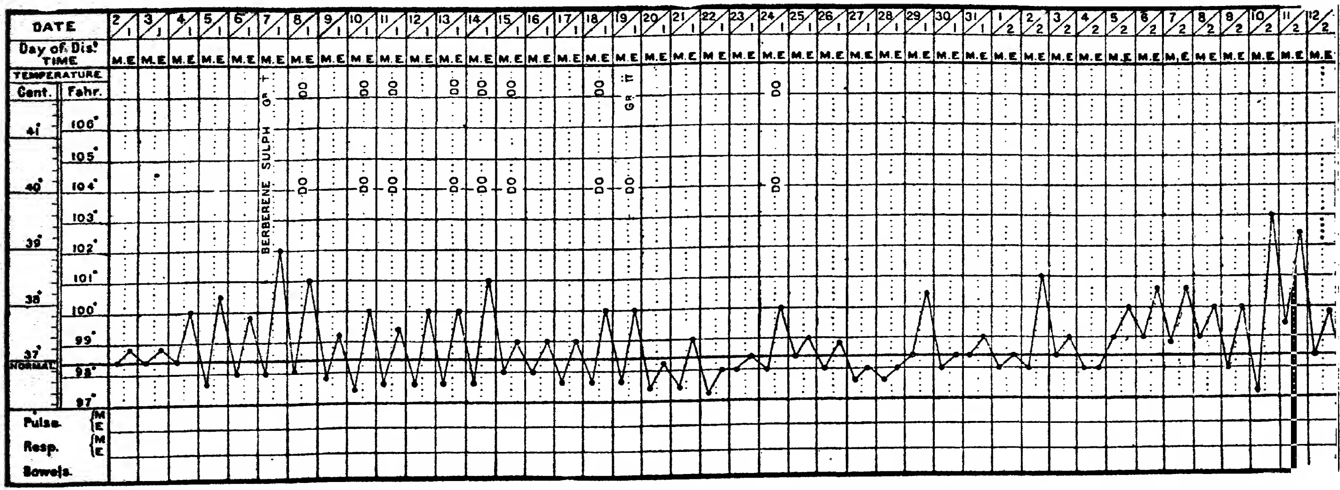
In another case, already mentioned, Abdul Aziz, narcotine was used intravenously. The effect on the blood was to some extent beneficial, as will be shown by the tables given under I, but there was no effect on the temperature. The patient was afterwards put on a course of intravenous injections of metallic antimony. Another case has been treated with the drug and so far the results are the same as in the above case.

It may be noted here that it is somewhat difficult to put narcotine into the veins as it is insoluble in water as well as in normal saline.

Doses of narcotine:—(1) $\frac{1}{5}$gr. given intravenously on 13-1-16; (2) $\frac{1}{5}$gr. on 14-1-16; (3) $\frac{1}{2}$gr. on 15-1-16; (4) 1 gr. on 17-1-16; (5) 1 gr. on 19-1-16; (6) 1 gr. on 21-1-16; (7) 1 gr. on 23-1-16; (8) 1 gr. on 26-1-16; (9) $1\frac{1}{2}$grs. on 31-1-16; (10) $1\frac{1}{2}$grs. on 2-2-16.

As just now stated, the effect of treatment with narcotine was only an improvement in the blood condition but there was no effect on the temperature and the L. D. bodies were present in the spleen.

The next alkaloid used was berberine sulphate. I have already reported that the combination of this drug with antimony does not seem to lead to better results than what




Temperature chart of a kala-azar case, treated with berberine sulph intravenously but discontinued as there was no improvement. (Para. 2, patient—Amiyo. Chart (*a*).

is obtained from antimony itself (*Indian Medical Gazette*, January, 1916). Two cases were treated with intravenous injections of this drug. One of them died of pneumonia and on *post mortem* examination L. D. bodies were found in the spleen. He was given five injections of berberine sulph. intravenously, the dose being ½ to 1½ grains.

The next patient, named Amiya, was at first treated with intravenous injections of berberine sulph. She was given altogether 10 injections. There was no improvement in the blood count, and there was no diminution in the size of the spleen and a very large number of L. D. bodies were present in the splenic blood after these injections. As the patient was not improving and was getting fever, berberine sulph. was discontinued. The drug was given in 1-grain doses for seven days and in 2-grain for three days.

*Result of Blood Examination*

R.B.C.—4,300,000, W.B.C.—2,400, Hb.—44% before treatment.

R.B.C.—4,000,000, W.B.C.—3,200, Hb.—46% after treatment with berberine sulphate.

The next alkaloid that has been used is bebeerine sulph. The effect of treatment with this drug has been unsatisfactory.

I have observed the following unpleasant effects after injections of sodium antimonyl tartrate or tartar emetic in some of my cases : (1) Rigor and temporary rise of temperature ; (2) pain in the gums ; (3) vomiting ; (4) a troublesome cough followed by vomiting in some cases ; (5) in one case there was an intense headache which lasted for nearly three days after injections of sodium antimonyl tartrate ; (6) diarrhœa.

*Conclusions*

(1) Colloidal metallic antimony has been used intravenously for the first time in kala-azar with very promising

results in the cases in which it was used. It is administered in very small doses.

(2) Antimony, in a state of fine subdivision, has been given intravenously with brilliant results in kala-azar.

(3) Tartar emetic and sodium antimonyl tartrate have also been used with marked benefit in the disease. The alternate administration of the two salts seems to be attended with the best results.

(4) The number of injections of metallic antimony required for a course of treatment is very much less than that of the soluble salts.

(5) Formaldehyde has been used in three cases with excellent results. The effects of eusol are much less marked than those of formaldehyde.

(6) Intravenous injections of narcotine is followed by an improvement in the condition of the blood. Intravenous injections of berberine sulph. and bebeerine sulph. have not given rise to any beneficial results in the cases in which they were tried.

(7) Antimony or its combination with formaldehyde will perhaps constitute a treatment in kala-azar and will cure a large percentage of cases of this fell disease which is deadlier than tuberculosis and kills hundreds of people in Assam and many parts of Bengal.

(8) If future observations confirm the view that three or four injections of metallic antimony are sufficient to bring about a complete and permanent cure of the disease, then we are in possession of a drug as powerful as quinine is for malaria, emetine for amœbic dysentery or salvarsan for syphilis. Its combination with formaldehyde will, perhaps, still more cut short the duration of the disease by the destruction of any antimony-fast parasites that may come into existence.

[Reprinted from the Indian Medical Gazette, Vol. LII, No. 9, Sept., 1917]

# FOURTH REPORT ON THE TREATMENT OF KALA-AZAR AND SOME BLOOD REACTIONS IN THIS DISEASE

## A

## FOURTH REPORT ON THE TREATMENT OF KALA-AZAR

Cases of enlarged spleen admitted into my wards from July to October in one year and whose splenic blood I examined numbered forty. Out of these, twelve showed the parasite of kala-azar in the splenic blood. These, together with a series of cases published by me in the *British Medical Journal* in May, 1908, show that out of one hundred and ninety cases of enlarged spleen in which the splenic blood was examined, the L. D. bodies were found in 72 of them or 38 per cent.

In another series of one hundred and sixty-six cases the results of examination of the splenic blood were as follows :—

(1) 27·1 per cent showed L. D. bodies.

(2) 21·1 per cent showed malarial parasites.

(3) ·012 per cent showed both malarial parasites and L. D. bodies.

(4) 51·8 per cent showed neither malarial parasites nor L. D. bodies.

Taking all the three series into account, it will be found that, out of three hundred and fifty-six cases in which the splenic blood was examined by me, only one hundred and eighteen or 33 per cent had the parasite of kala-azar,

The extreme rarity of malarial parasites being found with L. D. bodies is noteworthy and points to the conclusion that the two diseases rarely go together.

The results are also striking, in view of the fact that malaria and kala-azar cannot account for the enlargement of the spleen in a large percentage of the cases met with in the medical wards in Calcutta.

The cases with negative results in the examination of the splenic blood sometimes, though rarely, gave a blood picture resembling that of kala-azar. The notes of one such case are appended below :—

Patient, æt. 13, was admitted with the spleen extending $9\frac{1}{2}$ inches below the costal margin and with a history of fever for about two years. The spleen was punctured three times but neither any malarial parasites nor any L. D. bodies were discovered after very careful examination. The blood count was as follows :—

| | | |
|---|---|---|
| (1) R. B. C. | .. ... | 2,260,000 |
| (2) W. B. C. | ... ... | 1,500 |
| (3) Hb. | ... ... | 40 per cent. |

(4) $\frac{\text{W. B. C.}}{\text{R. B. C.}} = \frac{1}{1{,}540}$

Differential count :—

| | | |
|---|---|---|
| Polymorphonuclears | ... | 47 per cent. |
| Large mononuclears | ... | 13 ,, |
| Lymphocytes | ... | 36 ,, |
| Eosinophiles ... | ... | 4 ,, |

The temperature was normal throughout his stay in hospital, except on two occasions when the temperature rose to 103°F. The blood was examined on these occasions but neither malarial parasites nor L. D. bodies were discovered. The patient was in hospital for more than three months. Cases like the above are not infrequently met with.

From the above statistics, one is led to think of the possibility of an undiscovered, though common, cause of splenic enlargement in India. Are some of them cases of splenic anæmia or is it possible that there is an yet undiscovered phase of leishmania in which they disappear from the spleen and reside in some other parts of the body and in this quiescent stage give rise to little or no febrile manifestations, or are they due to some undiscovered parasites ?

The treatment of kala-azar described in this paper can be divided under the following heads :—

(1) Treatment with tartar emetic and sodium antimonyl tartrate given intramuscularly.

(2) Treatment with lithium antimonyl tartrate given intramuscularly.

(3) Treatment with aniline antimonyl tartrate given intravenously.

(4) Treatment with antimonious oxide in a state of fine subdivision given intravenously.

(5) Treatment with luargol.

(6) Treatment with colloidal oxide of antimony.

(7) Treatment with intravenous injections of bismuth tartrate solubilis.

Treatments under (1) to (5) have been described in detail in my treatise "*Kala-azar : Its Treatment*," published by Butterworth & Co. (India), Ltd. They will not, therefore, be described in detail here. The following conclusions can be drawn from them :—

(1) Cases treated with intramuscular injections of tartar emetic and sodium antimonyl tartrate improved remarkably. The injections were painful (dose 1 to 11 c.c. of 2 per cent solution given every 4th or 5th day).

(2) Cases treated with lithium antimonyl tartrate given intramuscularly showed much improvement in blood condition but patients left hospital before treatment was completed (dose ·1 to ·3 gramme). The injections were painful.

(3) Cases treated with aniline antimonyl tartrate, given intravenously, recovered completely (dose 1 to 10 c.c. of 2 per cent solution). The febrile reactions as well as vomiting, purging or rigors were much less common with this salt than with potassium or sodium antimonyl tartrate. I cannot, at present, state whether it is superior to sodium or potassium antimonyl tartrate in its therapeutic effects.

(4) Cases treated with intravenous injections of antimonious oxide in a state of fine subdivision were subsequently treated with metallic antimony and recovered completely (dose—·75 gr. to 1·5 grs.).

(5) Cases treated with luargol showed improvement in the blood condition, but the parasites persisted. Patients left hospital before treatment was completed.

(6) Cases treated with colloidal oxide of antimony.

In the *Addendum* to my *Treatise on Kala-azar* a reference has been made by me to the use of colloidal oxide of antimony. It seems to be the least toxic of all the antimonial preparations that I have used in kala-azar. It is at present difficult to compare its therapeutic value with that of other antimonial preparations. The following are the notes of one case treated with this drug :

Patient A—was admitted into my ward with the spleen extending 2½ inches below the costal arch. He was treated with intravenous injections of colloidal oxide of antimony, beginning with 4 c.c. of the colloid and increasing the dose by 1 c.c. at successive injections. For some time the injections were given every day. The patient has up to now had altogether eleven injections, the highest dose being 10 c.c. The effects of the treatment were as follows :—

Body weight—same.

Blood :—

R. B. C.—2,600,000, W.B C.—1,200, Hb.—40% on 19-4-17 before treatment.

R.B.C.—3,000,000, W.B.C.—2,400, Hb.—38% on 2-5-17 during treatment.

R.B.C.—3,300,000, W.B.C.—4,000, Hb.—48% on 12-6-17 during treatment.

There seems to be a steady but slow improvement in the general condition of the patient, and the drug seems to be perfectly free from any toxic symptoms.

The high temperature which the patient had before treatment came down, there being only a slight rise towards the evening, lasting for a very short period.

(7) Cases treated with intravenous injections of bismuth tartrate solubilis (bismuth sodium tartrate).

In a paper read before the Royal Society of Medicine, 9th January, 1909, Cushny pointed out that arsenic, antimony and bismuth killed trypanosomes in the concentration of 1 : 200,000. According to his observations bismuth proved too poisonous to the hcst and the damage done was irreparable.

I have given bismuth tartrate solubilis intravenously in a few cases of kala-azar. The toxic symptoms observed by Cushny in experimental trypanosomiasis were not noticed in any of my cases of kala-azar, and, in one case, there was such a remarkable improvement in the patient's condition that there was every chance of his perfect recovery. The preliminary notes of this case have been published in my *Treatise on Kala-azar* already referred to. I append here the full notes of this case, giving further observations since the publication of the above work.

Patient B—was admitted into my ward on the 4th February, 1917. The spleen extended 6 inches below the costal arch and L. D. bodies were found on spleen puncture. The patient was emaciated and cachectic. He was at first treated with one per cent solution of bismuth tartrate solubilis in distilled water, beginning with 1 c.c. and increasing up to 10 c.c. He was then treated with 5 c.c. of 2 per cent

solution, the dose being gradually increased to 9 c.c. Altogether 17 injections were given As a result of this treatment there had been a remarkable improvement in the condition of the patient as shown by the following note :—

(1) Increase in body weight—22 lbs. in two months, during which the treatment was continued.

(2) Size of the spleen—diminished by 4 inches.

(3) Disappearance of the fever.

(4) Marked improvement in the blood condition as shown by the following figures :—

R.B.C.—2,500,000, W.B.C.—3,800, Hb.—38% on 7-2-17 before treatment.

R.B C.—3,500,000, W.B.C.—2,600, Hb.—48%<br>
R.B.C.—4,000,000, W.B.C.—5,200, Hb.—50% } during treatment.

It will be seen that there was at first a diminution of the leucocytes during the treatment, but afterwards they steadily increased. The patient is now enjoying perfect health and has recently returned to work.

Two more cases were treated with the same drug, but they left hospital before treatment was completed. In one of these, the patient complained of intense pain in the gums each time the injections were given and there was marked pigmentation in the gums. The symptoms that are frequently met with after injections of antimonial preparations such as vomiting, cough, rigor, high fever and sudden syncope were completely absent in all these cases.

I hope to give at a future date a statement showing the comparative value of antimony and bismuth in the treatment of kala-azar.

## B

## A PRELIMINARY REPORT ON SOME BLOOD REACTIONS IN KALA-AZAR

I. The relative hæmoglobin-value of the resistant erythrocytes in kala-azar—

In the *Bio-Chemical Journal*, Vol. IV, 1909, I described a new method of testing blood in a paper entitled "The relative hæmoglobin-value of the resistant erythrocytes during hæmolysis of blood, etc." I have subsequently found that this factor is markedly diminished in kala-azar, as will be seen from the following figures :—

| | | 1 part of blood +2 parts of distilled water. |
|---|---|---|
| (1) | Healthy student ... ... | ·372 |
| (2) | Do. ... ... | ·434 |
| (3) | Do. .. ... | ·428 |
| (4) | Do. ... ... | ·450 |
| (5) | Do. ... ... | ·448 |
| | | Average = ·426 |
| (1) | Malarial fever ... ... | ·606 |
| (2) | Do. ... ... | ·303 |
| (3) | Do. ... ... | ·444 |
| | | Average = ·451 |
| (1) | Ankylostomiasis ... ... | ·714 |
| (2) | Do. ... ... | ·658 |
| | | Average = ·686 |
| (1) | Kala-azar ... ... | ·266 |
| (2) | Do. ... ... | ·250 |
| (3) | Do. ... ... | ·285 |
| (4) | Do. ... ... | ·142 |
| | | Average = ·236 |

The diagnostic importance of these facts can be settled by further observations.

II. The complement-deviation reaction in kala-azar—

A limited number of observations have been made by me in this direction. In a series of eight cases, the reaction was

found to be positive in six and negative in two. The diagnosis of each case was made by spleen puncture and the antigen used was made by the alcoholic extract of the fresh spleen of a kala-azar case made by grinding up one part of the spleen pulp with three parts of a mixture of equal parts of alcohol and ·85 per cent NaCl solution and then heated for an hour at 60°C. As the test originally employed by me was Fleming's modification of Wassermann test, and as Fleming's test is not free from fallacies, I do not consider any importance can be attached to these results till further observations have been made by more accurate methods. So far, these results differ from the observations of Pavoni in infantile kala-azar.

Very recently I tested this reaction according to the original Wassermann method and found that in only one out of four kala-azar cases this reaction was positive.

III. *Hæm-alkalinity.*—Archibald was the first to point out that the alkalinity of blood is diminished in kala-azar. Rogers and Shorten have confirmed this in Indian kala-azar.

I have tested the blood in a series of kala-azar cases for determining the basic reactivity. This was determined by a modification of that described by Moore and Wilson (*Bio-Chemical Journal*, 1909). Fifty c.mm. of blood were taken from the finger which was sterilized by 5 per cent formol solution and put into a perfectly dry sterilized tube and then quickly centrifuged. Ten c.mm. of the serum were treated with a solution of $\frac{N}{100}$ $H_2SO_4$ in a white porcelain shallow capsule, using a drop or two of a fresh dilution of an alcoholic solution of dimethyl-amido-azo-benzol in distilled water, the first indication of neutralization being given by a faint rose colour at the side of the liquid in the porcelain capsule. The dimethyl is first dissolved in alcohol and then one drop of this is mixed with distilled water just at the time of the experiment. A few drops of this are added to the serum to make it faint yellow.

In a series of cases, the average basic reactivity was found to be ·092 normal, as compared with ·178 normal in a series of healthy students (see my *Treatise on Studies in Hæmolysis*, Calcutta University Series).

IV. *Hæm-salinity.*—This was estimated by treating 10 c.mm. of the serum with $\frac{N}{100}$ $AgNO_3$ using a solution of $K_2CrO_4$ as an indicator. The average was found to be ·6538 per cent as contrasted with ·6654 per cent in a series of observations on healthy students.

V. It is frequently observed that when the blood of a kala-azar patient is mixed with excess of distilled water, a white flocculent precipitate forms. While this reaction is present in a large majority of cases of kala-azar, it has also sometimes been observed in other diseases, *e.g.*, phthisis, cancer of the liver, cirrhosis of the liver, chronic malaria, and cases of enlarged spleen in which no L. D. bodies were found on spleen puncture.

It has also been found that this precipitate is obtained by mixing the serum separated from the corpuscles, with excess of distilled water. The red corpuscles when dissolved in distilled water do not give rise to any such precipitate. The precipitate is soluble in a solution of sodium bicarbonate and also in dilute acetic acid, as well as in normal saline. It is insoluble in distilled water. It seems that the precipitate is of the nature of a globulin and is probably easily precipitated on account of the diminished alkalinity of the blood in kala-azar. Whether it is due to the presence of any excess of globulin in the serum of kala-azar cases, due to disintegration of leucocytes, or whether this globulin is of a specific nature, has not yet been determined. As regards its diagnostic importance, it may not be of great value because, as stated before, a similar precipitate has been found in other diseases.

One remarkable property of this globulin-like substance is that a solution of it in normal saline inhibits the action of the complement in a hæmolytic system.

My grateful thanks are due to Lt. Col. W. D. Sutherland, I.M.S., Imperial Serologist, and his assistant Dr. G. C. Mitter for providing me with materials for my hæmolysis work

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# ON THE PRESENCE OF AN EASILY PRECIPITABLE ANTI-COMPLEMENTARY GLOBULIN-LIKE SUBSTANCE IN HUMAN SERUM AND ITS IMPORTANCE IN THE DIAGNOSIS OF KALA-AZAR

When human serum is diluted with excess of distilled water it becomes cloudy owing to partial precipitation of the serum globulin. Under certain circumstances, a copious precipitate forms instead of mere cloudiness. This precipitate is due to a globulin-like substance as is evident from the following facts :—

(1) It is soluble in normal saline, in dilute acids, and in dilute sodium bicarbonate solution. It is also soluble in sodium hydrate solution.

(2) It is precipitated from its solution in normal saline when the solution is treated with equal parts of a saturated solution of $(NH_4)_2SO_4$ or when it is saturated with $MgSO_4$ or NaCl.

(3) It is not precipitated by $NH_4OH$ from its solution in dilute acids.

(4) It is insoluble in distilled water.

On chemical analysis, this substance is found to contain C, N, H, O, but so far I have not been able to detect in it the presence of S, P, or any halogen. After being thoroughly washed in distilled water it can be collected as a white precipitate having a granular appearance under the microscope.

If further investigations confirm the observation that this substance does not contain any phosphorus or sulphur, then it will be found to be different from serum-globulins in chemical composition.

I hope to enter, at a future date, into the chemical nature of this substance and at present shall content myself by assuming that it is globulin-like in nature.

In the *Indian Medical Gazette*, September, 1917, I pointed out that this copious precipitate is frequently observed when the serum of a kala-azar case is mixed with excess of distilled water. I also pointed out that a precipitate apparently similar to this has sometimes been observed in other diseases, *e.g.*, chronic malaria, phthisis, cancer of the liver, etc. I was not, therefore, then able to state whether the presence of this precipitate was of any diagnostic importance in kala-azar. Further observations lead to the conclusion that, if instead of using an excess of distilled water (which in my original experiments consisted of 15 to 20 times the amount of serum used) one uses two or three volumes of distilled water then the precipitate appears almost exclusively in kala-azar. Thus in a series of 20 cases of kala-azar, the following results were obtained :—

One part of serum plus two parts of distilled water produced a copious precipitate. In some cases one part of serum plus one and-a-half parts of distilled water gave rise to a distinct precipitate.

Similar experiments were made with the serum of a series of cases suffering from other diseases and a negative result was always obtained.

| | | One part of serum plus two parts of distilled water |
|---|---|---|
| (1) | Phthisis ... ... | No pp. |
| (2) | Malarial fever ... ... | No pp. |
| (3) | Cirrhosis of the liver .. ... | No pp. |
| (4) | Enteric fever ... ... | No pp. |
| (5) | Bright's disease ... ... | No pp. |
| (6) | Ankylostomiasis ... ... | No pp. |
| (7) | Pernicious anaemia ... ... | No pp. |
| (8) | Dengue ... ... | No pp. |
| (9) | Dysentery ... ... | No pp. |
| (10) | Pneumonia ... .. | No pp. |
| (11) | Catarrhal jaundice ... ... | No pp. |
| (12) | Broncho-pneumonia with enlarged spleen (no L. D. bodies in the spleen) ... ... | No pp. |

In a few cases with enlarged spleen in which no L. D. bodies were found on spleen puncture, a similar precipitate was obtained, though clinically they looked like kala-azar.

Whether these are cases of kala-azar in which the parasites could not be found on spleen puncture, as sometimes is the case as pointed out by Leishman, or whether some of them are cases of spontaneous cure from kala-azar, cannot be definitely stated in the present state of our knowledge.

I have also found that if distilled water is gently poured on the top of the serum of a kala-azar case, a distinct white ring is formed at the junction similar to the ring of albumin that is found on addition of nitric acid to a solution of albumin. This test also appears to be of diagnostic importance in kala-azar. A similar ring is also observed in some obscure cases of enlarged spleen mentioned before.

To perform the above two tests proceed as follows :—

(1) Two c.c. of the blood from a prominent vein of a kala-azar case are drawn by a glass syringe and the blood quickly centrifuged. The serum freed from the clot is

introduced into a miniature test tube with a capillary pipette and then a small amount of distilled water is gently poured over the serum. A distinct *white ring* forms at the junction in every case of kala-azar. I propose to call this the "*globulin ring test*" of kala-azar.

(2) The serum is collected in a miniature test tube and then mixed with two or three parts of distilled water. A white precipitate forms in every case of kala-azar. I propose to call this the "*globulin precipitation test*" of kala-azar.

### *The Anticomplementary Properties of the Globulin-like Substance*

This fact was briefly touched upon by me in the September number of the *Indian Medical Gazette*. The following is a detailed method of showing this remarkable property of this globulin-like substance :—

One c.c. of the serum of a kala-azar case is mixed with fifteen c.c. of distilled water. The precipitate is collected, washed thoroughly with distilled water and then dissolved in one c.c. of normal saline. The following observations were made :—

(1) Take, for instance, a hæmolytic system in which the following are the doses of the component parts—

·15 c.c. of anti-sheep amboceptor + ·5 c.c. of guinea-pig's complement + ·5 c.c. of sheep's corpuscles = complete hæmolysis.

(2) (*a*) Mix ·5 c.c. of guinea-pig's complement with ·2 c.c. of the solution of the above precipitate in normal saline and incubate for half an hour.

(*b*) Add to this ·15 c.c. of anti-sheep amboceptor + ·5 c.c. of sheep's erythrocytes = no hæmolysis (I propose to call this test the "*anticomplementary globulin test*" of kala-azar).

(3) 1 c.c. of the serum of the same kala-azar case + ·5 c.c. of sheep's erythrocytes = complete hæmolysis (due, no

doubt, to the natural complement and amboceptor frequently present in human serum).

(4) (*a*) Heat the serum to 55°C. for half an hour.

(*b*) Add ·2 c.c. of the heated serum to ·5 c.c. of guinea-pig complement and incubate for half an hour.

(*c*) Add ·5 c.c. of sheep's erythrocytes to (*b*) and incubate = complete hæmolysis.

From the above the following conclusions are drawn :—

1. One part of serum of a kala-azar case with two or three parts of distilled water gives a distinct precipitate. Such a precipitate is not obtained in any other disease except kala-azar and some rare obscure cases of enlarged spleen. Its presence is, therefore, of much diagnostic importance (*globulin precipitation test*).

2. Gently pour distilled water on to the top of the serum of a kala-azar case, a distinct white ring forms at the junction. This ring is not observed in any other disease except kala-azar and some rare obscure cases of enlarged spleen. Its presence is, therefore, of much diagnostic importance (*globulin ring test*).

3. The solution in normal saline of the above globulin-like substance present in the serum of kala-azar patients inhibits the action of the complement in a hæmolytic system consisting of sheep's corpuscles, anti-sheep amboceptor and guinea-pig's complement (*anticomplementary globulin test*).

4. This globulin-like substance does not inhibit the action of the natural complement normally present in the serum as long as it is not separated from the serum by the action of distilled water.

5. This globulin-like substance is probably in combination with some constituents of the serum, and as long as this combination exists, it exerts no anticomplementary action. Distilled water also breaks up this combination. It is not broken up by heating the serum to 55°C.

6. We have regarded the above substance to be globulin-like in nature, but further investigations will be required to determine its chemical nature. At present it seems to differ from serum-globulin in not containing S or P.

7. Globulin-like substances are sometimes precipitated from the serum of cases suffering from chronic malaria, cancer of the liver, etc., by the addition of excess (15 or 20 parts) of distilled water, as has been pointed out by me in the *Indian Medical Gazette*, September, 1917. The precipitate obtained is much greater than, and must not be confounded with, what appears as a cloudiness when normal serum is diluted with excess of distilled water, due to partial precipitation of serum globulin. None of these, however, are precipitated when the serum is diluted with only *two* or *three* parts of distilled water. The properties of these globulin-like substances will form the subject of a future investigation. It cannot, at present, be stated whether they possess any anticomplementary properties similar to what is shown by the globulin-like protein separated from the serum of kala-azar patients. I cannot also state whether they contain any P or S.

8. It is possible that in different diseases, globulin-like proteins, perhaps of a specific nature, are present in the serum with varying degrees of solubility in salt solution. The one present in kala-azar is characterised by being very easily precipitated by addition of a small amount of distilled water to the serum. This is evidently due to the inability of NaCl in the diluted serum to hold the protein in solution.

Whether the *anticomplementary globulin test*, the *globulin precipitation test* and the *globulin ring test* are absolutely pathognomonic of kala-azar can only be settled by further investigations, but, so far, they seem to be very valuable tests in the diagnosis of the disease. In some cases of very slightly enlarged spleen, the production

of these reactions led to the diagnosis of kala-azar, which was afterwards confirmed by spleen puncture. In making the ring test the serum must first be diluted ten to twenty times with normal saline.

My grateful thanks are due to Lt. Col. R. P. Wilson, I.M.S., for giving me every facility in carrying on my researches in the Campbell Hospital. I am also deeply indebted to Col. W. D. Sutherland, I.M.S., and his assistant, Dr. G. C. Mitter, for providing me with materials for conducting the serological portion of this investigation.

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# TREATMENT OF KALA-AZAR WITH INTRAMUSCULAR INJECTIONS OF HYPER-ACID ANTIMONYL TARTRATE (+ URETHANE)

Since the discovery of antimony as a specific in the treatment of kala-azar, attempts have been made to discover a preparation which could be given intramuscularly without local reaction. The ordinary antimonyl preparations, such as tartar emetic or sodium antimonyl tartrate, give rise to violent local reaction and cannot therefore be used intramuscularly.

Caronia has used acetyl-*p*-aminophenyl-stibinate of sodium intramuscularly in the treatment of infantile kala-azar with good results and subsequently it was used by Kharina-Marinucci.

In seeking for a preparation of antimony which will give little local irritation, we should use one which will be quickly absorbed without dissociation or decomposition. Such a preparation I have found in hyper-acid antimonyl tartrate (+ urethane). It is readily soluble in water, stable in aqueous solution for indefinite periods, and is quickly absorbed without decomposition after intramuscular injection. As urethane is not a base, it probably remains in solution with the antimonyl compound in the form of a mixture.

Experiments are being conducted by me to determine its toxic dose as compared with its curative dose, and, so far as I have been able to determine, it appears to be the least toxic of all the antimonial preparations and its curative

dose seems to be much smaller than that of other antimonial preparations. Further observations on this subject will be communicated in a future paper.

The following are the series of the first four successive cases which have been treated successfully with this compound. In each of these cases the diagnosis was made by the presence of L. D. bodies in the spleen and the cure was shown by their disappearance therefrom :—

1. Patient B. S.—was admitted into my ward on 25-9-19, with the spleen extending 6 in. below the costal margin in the left nipple line. He was given intramuscularly $2\frac{1}{2}$ c.c. of a two per cent solution of the hyper-salt with urethane. Altogether 14 injections were given from twice to four times a week. The results of treatment were as follows :—

R.B.C.—2,800,000, W.B.C.—1,800, Hb.—46 per cent on 26-9-19 before treatment.

R.B.C.—4,700,000, W.B.C.—13,800, Hb.—60 per cent on 5-1-20 after treatment.

There was a marked increase in weight, the spleen could not be felt below the costal arch, no L. D. bodies could be found on spleen puncture and the fever subsided.

2. Patient M.—was admitted into my ward on 23-8-19, the spleen extending 5 in. below the costal margin in the left nipple line. He was given $2\frac{1}{2}$ to 5 c.c. of 2 per cent solution of the hyper-salt with urethane intramuscularly. Altogether 15 injections were given from twice to four times a week. The results of treatment were as follows :—

R.B.C.—3,300,000, W.B.C.—2,200, Hb.—38 per cent on 8-9-19 before treatment.

R.B.C.—4,600,000, W.B.C.—16,000, Hb.—60 per cent on 23-12-19 after treatment.

There was a marked increase in weight, the spleen could just be felt below the costal margin, no L. D. bodies could be found on spleen puncture and the fever subsided.

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# A PRELIMINARY NOTE ON THE GLOBULIN, ALBUMIN AND CHOLESTEROL CONTENTS OF THE BLOOD IN KALA-AZAR

In the *Indian Medical Gazette*, 1917, and in the *Treatise on Kala-azar by Brahmachari* (*2nd Edition, 1920*) it was pointed out that the copious precipitate that was found when distilled water was added to kala-azar serum was globulin-like in nature and it was suggested that this might be due to an easily precipitable globulin or excess of globulin in kala-azar blood. We have since estimated the globulin content of the blood in some cases of kala-azar and the following results have been obtained. The diagnosis in each case was made by the presence of L. D. bodies in the splenic blood :—

*First Case.*

| | | | |
|---|---|---|---|
| Globulin | ... | ... | 1·9 per cent. |
| Albumin | ... | ... | 1·2 ,, |

*Second Case.*

| | | | |
|---|---|---|---|
| Globulin | ... | ... | 1·86 per cent. |
| Albumin | ... | ... | 1·03 ,, |

*Third Case*

| | | | |
|---|---|---|---|
| Globulin | ... | ... | 2·1 per cent. |
| Albumin | ... | ... | 1·72 ,, |

*Fourth Case.*

| | | | |
|---|---|---|---|
| Globulin | ... | ... | 1·9 per cent. |
| Albumin | ... | ... | 1·36 ,, |

In a series of healthy Indians the following results were obtained :—

| | | |
|---|---|---|
| Total protein ... | ... | 4·9 to 6·9 per cent |
| Albumin ... | ... | 3·5 to 4·2 ,, |
| Globulin ... | ... | 1·0 to 2·7 ,, |

The following figures have been obtained by other observers : —

*Howel :*

| | | |
|---|---|---|
| Total protein ... | ... | 6·014 to 7·6 per cent |
| Albumin ... | ... | 4·52 p.c. |
| Globulin ... | ... | 3·1 p.c. |

*McLeod :*

| | | |
|---|---|---|
| Total protein ... | ... | 6·7 to 8·7 p.c. |
| Albumin ... | ... | 4·95 to 7·7 p.c. |
| Globulin ... | ... | 1 to 2·54 p.c. |

### *Cholesterol content of the Precipitate obtained by Diluting Blood with Excess of Distilled Water*

After diluting a volume of blood with 50 parts of distilled water and extracting the precipitate formed with ether, alcohol, chloroform and amyl alcohol, the following figures were obtained :—

| | | |
|---|---|---|
| *Normal blood* ... | ... | ·14 to ·22 per cent. |
| *Kala-azar blood :* | | |
| 1st case ... | ... | ·38 per cent. |
| 2nd ,, ... | ... | ·29 ,, |
| 3rd ,, ... | ... | ·43 ,, |
| 4th ,, ... | ... | ·46 ,, |
| | Average | ·39 per cent. |

It will be seen that while, in kala-azar, the total protein and albumin contents of the blood are diminished, the cholesterol content in the precipitate obtained by diluting the blood with 50 parts of distilled water and the proportion of globulin to albumin contents are increased.

We have observed that when kala-azar blood is mixed with distilled water, the precipitate formed shows the presence of many red corpuscles under the microscope. The precipitate, in our opinion, consists partly of globulin and partly of walls of red corpuscles. Though most of the red corpuscles present in the precipitate are nothing but shadow corpuscles, we have still to determine whether the excess of cholesterol present in kala-azar blood interferes with the hæmolysing effect of distilled water upon the red corpuscles in kala-azar blood and this will form the subject of a future investigation.

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# THE TREATMENT OF KALA-AZAR WITH SOME NEW ANTIMONIAL PREPARATIONS

The new antimonial compounds which I am going to describe in this paper may be divided into two classes :—

(1) New antimonyl tartrates.

(2) Aryl or phenyl antimonial compounds.

(1) includes (*a*) urea antimonyl tartrate and (*b*) ammonium antimonyl tartrate.

(2) includes (*a*) phenyl-stibinate of sodium, (*b*) acetyl-*p*-amino-phenyl stibinate of sodium and (*c*) *p*-amino-phenyl-stibinate of sodium or antimony analogue of soamin.

I shall now give a report of my experience with some of these antimonial compounds in the treatment of kala-azar. As most of these compounds have only been recently used by me in this disease, the report must be regarded as a preliminary one.

## *I. (a) Urea Antimonyl Tartrate*

This is a new compound. The method of its preparation was described by me at the July meeting of the Asiatic Society of Bengal, 1920 and published in the *Journal and Proceedings of the Society*, Vol. XVI, 1920. The amount of antimony present in it is nearly 38 per cent. It has been used by me both intravenously and intramuscularly. Intravenously it has, up to now, been used in four cases.

The following are the notes of these cases :—

(1) Patient K., æt. 35. Leishman-Donovan bodies present in the spleen. 2 to 5 c. c. of 2 per cent solution injected.

*Result of Treatment.*—Blood : (1) Red blood corpuscles—2,400,000 ; white blood corpuscles—1,000 ; hæmoglobin—38 per cent on July 16, 1920, before treatment. (2) Red blood corpuscles—3,000,000; white blood corpuscles—5,800 ; hæmoglobin—44 per cent, on November 13, 1920, after treatment. Spleen extended 5½ in. below the costal arch on July 16, 1920, before treatment, and 2½ in. below the costal arch on November 13, 1920, after treatment. Increase of body weight, 7 lbs. Fever stopped after twelve injections. No Leishman-Donovan bodies found in the spleen after fifteen injections.

(2) Patient S., æt. 15. Leishman-Donovan bodies present in the spleen. 2 to 4 c.c. of 2 per cent solution injected.

*Result of Treatment.*—Blood : (1) Red blood corpuscles—3,600,000 ; white blood corpuscles—4,000 ; hæmoglobin, 46 per cent before treatment. (2) Red blood corpuscles—3,900,000; white blood corpuscles—10,400; hæmoglobin—52 per cent, on November 1, 1920, after seventeen injections. Spleen extended 5 in. below the costal arch at the beginning of treatment and 1½ in. below the costal arch after seventeen injections. No Leishman-Donovan bodies found on spleen puncture after completion of treatment. Increase in body weight, 1 stone. Fever stopped after eight injections.

(3) Patient H., æt. 20. Leishman-Donovan bodies present in the spleen. 2 to 4 c.c. of 2 per cent solution injected.

*Result of Treatment.*—Blood : (1) Red blood corpuscles—2,400,000; white blood corpuscles, 1,600; hæmoglobin—32 per cent before treatment. (2) Red blood corpuscles—

3,500,000; white blood corpuscles—10,000; hæmoglobin—52 per cent on November 13, 1920, after sixteen injections. Spleen extended $5\frac{1}{4}$ in. below the costal arch before treatment and $1\frac{1}{2}$ in. below the costal arch after sixteen injections. No Leishman-Donovan bodies found on spleen puncture after completion of treatment. Increase of body weight, 2 lbs. Fever stopped after three injections.

(4) Patient K., æt. 26, Leishman-Donovan bodies present in the spleen. 2 to 6 c.c. of a 2 per cent solution injected.

*Result of Treatment.*—Blood: (1) Red blood corpuscles—2,400,000; white blood corpuscles—1,200; hæmoglobin—38 per cent before treatment. (2) Red blood corpuscles—2,700,000; white blood corpuscles—3,400; hæmoglobin—42 per cent after eleven injections. Spleen extended 5 in. below the costal arch before treatment and 4 in. below the costal arch after eleven injections.

Up to now, urea antimonyl tartrate does not seem to be superior to tartar emetic or antimonyl sodium tartrate, but it appears to me that symptoms, such as vomiting, severe cough or high rise of temperature do not follow the intravenous injections of urea antimonyl tartrate.

Intramuscularly this preparation has up to now been used in two cases.

The following are the notes of these cases:—

(1) Patient S., æt. 24. Leishman-Donovan bodies found on spleen puncture.

*Result of Treatment.*—Blood: (1) Red blood corpuscles—1,400,000; white blood corpuscles—1,600; hæmoglobin—22 per cent before treatment. (2) Red blood corpuscles—2,300,000; white blood corpuscles—4,800; hæmoglobin—32 per cent after eleven injections. (3) Red blood corpuscles—3,300,000; white blood corpuscles—7,800; hæmoglobin—40 per cent after twenty injections. Spleen $4\frac{1}{2}$ in. below the costal arch before treatment and 3 in. below

the costal arch after eleven injections and 1 in. after twenty injections. Body weight same as before. No Leishman-Donovan bodies found on spleen puncture after twenty injections. Dose—1 to 2 grs. daily. There was some inflammation but never any suppuration at the sites of injection.

(2) Patient D., æt. 10. Leishman-Donovan bodies present in the spleen.

*Result of Treatment.*—Blood : (1) Red blood corpuscles—2,800,000; white blood corpuscles—3,800; hæmoglobin—30 per cent before treatment. (2) Red blood corpuscles—3,600,000; white blood corpuscles—3,400; hæmoglobin—42 per cent after twelve injections. (3) Red blood corpuscles—3,600,000; white blood corpuscles—4,400; hæmoglobin—48 per cent after twenty injections. Size of spleen reduced by 1 in. Dose—$\frac{1}{2}$ to 1 gr. Local reaction—no abscess but sometimes swelling and inflammation. Fever stopped after sixteen injections. No Leishman-Donovan bodies found on spleen puncture after sixteen injections.

In both the above cases the local reactions were less severe than what are met with in the case of potassium antimonyl tartrate.

### (*b*) *Ammonium Antimonyl Tartrate*

This preparation has up to now been used by me only intramuscularly. The amount of antimony present in it is nearly 40 per cent. I have prepared this salt by neutralizing hyper-acid antimonyl tartrate with ammonium carbonate and washing the precipitate with absolute alcohol.

The following are the notes of one case treated with it intramuscularly :—

Patient B., æt. 10. Leishman-Donovan bodies present in the spleen. (1) Red blood corpuscles—1,600,000; white blood corpuscles—800; hæmoglobin—36 per cent before

treatment. (2) Red blood corpuscles—2,400,000; white blood corpuscles—2,800; hæmoglobin—40 per cent after three injections. Spleen 5 in. below the costal arch before treatment and 3 in. after three injections. Dose—1 to 2 c.c. of 2 per cent solution. Fever stopped after three injections Treatment still being continued.

Intramuscularly this salt is less irritating than Tzuki's antiluetin, which is ammonium potassium antimonyl tartrate.

*N.B.*—It is interesting to note that the leucocyte count was so low as 800 before commencement of treatment.

### *II. Aryl Antimonial Compounds*

I have been successful in preparing these compounds with the help of my chemist, who has been working under me under a grant from the Indian Research Fund Association.

The following compounds have already been made :—

(*a*) Phenyl-stibinic acid and its sodium and ammonium salts.

(*b*) *p*-Amino-phenyl-stibinic acid and its sodium salt.

(*c*) Acetyl-amino-phenyl-stibinic acid and its sodium salt.

(*a*) The salts of phenyl-stibinic acid are too irritating and too toxic to be used for therapeutic purposes.

(*b*) The amino-aryl compounds are extremely difficult to prepare. Stibenyl, which is allied to the acetyl compound, has been used by me in two cases, in one intramuscularly, and in the other intravenously.

The following are the notes of these cases. The treatment is still being continued.

(1) Patient M., æt. 35. Leishman-Donovan bodies found in the spleen. (1) Red blood corpuscles—2,100,000;

white blood corpuscles—2,800 ; hæmoglobin—32 per cent before treatment. (2) Red blood corpuscles—2,600,000 ; white blood corpuscles—2,200 ; hæmoglobin—34 per cent after six injections. (3) Red blood corpuscles—3,400,000 ; white blood corpuscles—8,200 ; hæmoglobin—48 per cent after eleven injections. Injections given intravenously on alternate days. After the ninth injection patient developed eruptions on his body similar to chicken-pox. Spleen could just be felt below the costal arch. Increase of body weight, 2 stone. No Leishman-Donovan bodies on spleen puncture. Doses—(1) ·1 grm., (2) ·15 grm., (3) ·2 grm., (4) ·3 grm., (5) 4 grm., (6) ·5 grm., (7) ·6 grm., (8) ·8 grm., (9) 1 grm., (10) 1·5 grms., (11) 2 grms.

(2) Patient H., æt. 19. Leishman-Donovan bodies found in the spleen. (1) Red blood corpuscles—2,900,000 ; white blood corpuscles—1,800 ; hæmoglobin—54 per cent before treatment. (2) Red blood corpuscles—3,900,000 ; white blood corpuscles—2,800 ; hæmoglobin—50 per cent after five injections. Doses—(1) ·1 grm., (2) ·15 grm., (3) ·2 grm., (4) ·3 grm., (5) ·8 grm. All the doses were given intramuscularly.

The injections were given on alternate days. There was much local irritation with pain and effusion into the injected parts which slowly subsided.

This patient also developed eruptions similar to the above after the last injection.

(c) *p-Amino-phenyl-stibinate of sodium* (antimony analogue of soamin).—This has been used by me in one case intramuscularly in 1-grain doses given every day. No local reaction. I propose to give it the name of Stibamine. Subsequently I have been using it in bigger doses of ·2 to ·3 grm., as its toxicity appears to be low.

So far, it is too early to give any definite opinion about the effect of this antimonial preparation.

The best antimonial preparation to be used in the treatment of kala-azar has not yet been discovered. Tartar emetic and antimonyl sodium tartrate have their serious drawbacks, with which unfortunately we are more or less familiar. The discovery of the amino antimony analogues of arsenical compounds opens up a new vista in the treatment of the disease. Anyone who thinks that the last word about the best antimony preparation has already been told in tartar emetic or antimonyl sodium tartrate is wrong. One must pass from one antimony preparation to another to discover the one that is best. It is stated that Ehrlich used nearly 600 arsenical preparations in 900 days from 1910-1913 in his attempt to discover the best one for the treatment of spirillosis. And still we hear of newer arsenical compounds, such as methylated salvarsan compounds, hexamino-arseno-benzene, triamino-phenyl arsenic acid, silver-salvarsan and others. Something approaching this has just been begun in the case of antimony.

To me it appears that a day will come when in studying the organic derivatives of antimony one will be reminded of a simile employed by Dr. Bertheim about the chemistry of organic arsenical compounds. He compares it to a sleeping beauty slumbering until, quite recently, in an unfrequented corner of *Beilstein*, but who, now awakened, appears as one of the fairy gifts which synthetic chemistry bestows from time to time upon mankind. Let us, who have to deal with dreadful kala-azar, hope that such a fairy will be discovered in the case of antimony. The words of Basil Valentine, who stated centuries ago that he who deals with antimony must have an ample mind, are very true.

May I incidentally refer here to the leucocyte-increasing property of narcotine when given intravenously in solution in tartaric acid. This has already been noted by me.

The two following cases of kala-azar illustrate the leucocyte-increasing properties of narcotine :—

(1) Patient M., æt. 12. Leishman-Donovan bodies present in the spleen. (1) Red blood corpuscles—3,200,000; white blood corpuscles—3,400; hæmoglobin—42 per cent before treatment. (2) Red blood corpuscles—3,400,000; white blood corpuscles 7,600; hæmoglobin—48 per cent after twenty-two injections Condition of spleen, same as before. Increase of body weight—5 lbs. in three weeks. The increase of the leucocytes does not seem to be temporary. Dose—$\frac{1}{4}$ to 1 gr., given every day.

(2) Patient A., æt. 30. Leishman-Donovan bodies found in the spleen. (1) Red blood corpuscles—2,400,000; white blood corpuscles—2,400; hæmoglobin—46 per cent before injection. (2) Red blood corpuscles—3,000,000; white blood corpuscles—5,200; hæmoglobin—46 per cent after twenty-six injections.

I have been able to prepare a new compound of narcotine antimonyl tartrate as a definite crystalline substance. It is sparingly soluble in water but easily soluble in tartaric acid.

*Remarks*

(1) Urea antimonyl tartrate, a new definite compound, has been prepared and used in kala-azar intravenously as well as intramuscularly.

(2) Ammonium antimonyl tartrate has been prepared in a pure state. It is less irritating than antiluetin. It has been used intramuscularly in kala-azar.

(3) Narcotine antimonyl tartrate, a new compound, has been prepared. Its leucocyte-increasing property has been noted.

(4) Acetyl-*p*-amino-phenyl-stibinate of sodium, which is allied to the patented Stibenyl, has been prepared and used in kala-azar.

(5) *p*-Amino-phenyl-stibinate of sodium, which is the antimony analogue of soamin, has been prepared, and is being used in kala-azar. Its toxicity appears to be low. I propose to give it the name Stibamine.

(6) As the best antimonial preparation for the treatment of kala-azar has yet to be discovered, one must not rest contented with the use of tartar emetic or sodium antimonyl tartrate.

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# THE GLOBULIN OPACITY TEST IN KALA-AZAR

Two simple serum tests for kala-azar were described by Brahmachari (*Indian Medical Gazette*, December, 1917) and were named as (1) the globulin precipitation test and (2) the globulin ring test. The former consists in mixing one part of serum with two parts of distilled water when a distinct precipitate forms in the case of kala-azar serum. The latter consists in adding a few drops of distilled water on to the top of serum from a kala-azar patient when a distinct turbidity forms at the top of the serum. These tests have recently been confirmed by Milo, working in the University of Messina, and by some observers in China.

That the above precipitate is a globulin is proved by the following tests :—

(1) It is soluble in normal saline, in dilute acids, in sodium bicarbonate solution and in sodium hydroxide solution.

(2) It is precipitated from its solution in normal saline when the solution is treated with equal parts of saturated solution of $(NH_4)_2SO_4$ or when it is saturated with $MgSO_4$ or NaCl.

(3) It is not precipitated by $NH_4OH$ from its solution in dilute acids.

(4) It is insoluble in distilled water.

It has been subsequently found that if the globulin obtained by treating one part of serum with two parts of distilled water be dissolved in the serum of an individual on which formaldehyde has no action, an opacity is obtained if a drop of formaldehyde is added to it. If the same globulin is dissolved in normal saline, it also gives rise to an opacity or precipitate when formaldehyde is added to the solution, especially when the solution is rendered faintly alkaline by the addition of a little sodium bicarbonate. This opacity, however, is generally less than that which is obtained when formaldehyde is added to the original serum. It is probably due to electrolytes other than sodium chloride being present in the serum. There is no doubt that this easily precipitable globulin which was described some time ago in kala-azar serum by Brahmachari is responsibile for the aldehyde test.

### *The Globulin Opacity Test*

By estimating quantitatively the amount of water-precipitable globulins present in a serum, we have succeeded in discovering a test which we propose to call the *globulin opacity test* for kala-azar. The test is carried out as follows :—

One part of serum is mixed with 6 parts of distilled water when a turbidity forms. The precipitated globulin, after being uniformly mixed with the diluted serum, is poured into a graduated cylinder, the diameter of which is one inch. On looking through the height of the fluid containing the precipitated globulin over some black spots fixed to the bottom of the cylinder and adding more and more of the fluid till the spots become just invisible, a point is reached which gives an estimate of the globulin precipitated.

We have observed that in kala-azar a value is obtained which is fairly diagnostic of the disease, as will be seen in the following table.

| | | Precipitated globulin (1 part of serum + 6 parts of $H_2O$). Height in inches at which the black spots disappear. |
|---|---|---|
| Kala-azar | ... | 1·1 |
| Do. | ... | 1·4 |
| Do. | ... | 1·6 |
| Do. | ... | 0·7 |
| Do. | ... | 1·3 |
| Do. | ... | 0·75 |
| Do. | ... | 1·25 |
| Do. | ... | 0·75 |
| Do. | ... | 0·9 |
| Do. | ... | 0·85 |
| Do. | ... | 0·9 |
| Do. | ... | 1·25 |
| Do. | ... | 1·25 |
| Do. | ... | 1·25 |
| Malarial fever | ... | 3·25 |
| Do. | ... | 3 |
| Typhoid fever | ... | 3·5 |
| Do. | ... | 3 |
| Pneumonia | ... | 2 |
| Nephritis | ... | 4·2 |
| Aneurism | ... | 5·4 |
| Hemiplegia | ... | 5 |
| Healthy student | ... | above 8 |
| Do. Do. | ... | do. 10 |
| Do. Do. | ... | do. 10 |
| Do. Do. | ... | do. 10 |
| Do. Do. | ... | do. 10 |
| Do. Do. | . | do. 10 |
| Do. Do. | ... | ... 6·5 |

*N.B.*—The height in inches at which the figures disappeared is *inversely proportional* to the amount of the globulin present in the serum.

*From the above we may conclude that if in any case the height of globulin precipitated by diluting the serum with 6 parts of distilled water and estimated in the above way is 1·25 in. or less, it may be regarded as fairly diagnostic of kala-azar.*

We have also discovered that the total amount of water-precipitable globulins present in kala-azar is greater than that generally found in health or in other diseases. The total water-precipitable globulins are obtained by diluting one part of the serum with 200 parts of distilled water and estimating it in the same way as above.

The following table gives the value of the total water-precipitable globulins in certain diseases.

| | | | Precipitated globulin (1 part of serum + 200 parts of $H_2O$) Height in inches at which the black spots disappear. |
|---|---|---|---|
| Phthisis | ... | ... | 3·1 |
| Do. | ... | ... | 3·9 |
| Do. | ... | ... | 3·15 |
| Kala-azar | ... | ... | 1·9 |
| Do. | ... | ... | 1·45 |
| Do. | ... | ... | 1·6 |
| Do. | ... | ... | 1·75 |
| Do. | ... | ... | 1·9 |
| Do. | ... | ... | 1·5 |
| Do. | ... | ... | 1·8 |
| Do. | ... | ... | 1·35 |
| Do. | .. | ... | 1·65 |
| Do. | ... | ... | 1·4 |
| Do. | ... | ... | 1·65 |
| Do. | ... | ... | 1·25 |
| Do. | ... | ... | 1·7 |
| Do. | ... | ... | 1·35 |
| Enlarged spleen (not leishmaniasis) | | ... | 2·5 |
| Dysentery | ... | ... | 2·7 |
| Do. | ... | ... | 2·5 |
| Chronic dysentery | ... | ... | 5·5 |
| Liver abscess with broncho-pneumonia | | | 2·9 |

| | Precipitated globulin (1 part of serum+200 parts of $H_2O$). Height in inches at which the black spots disappear. |
|---|---|
| Broncho-pneumonia with enlarged spleen | 4·4 |
| Broncho-pneumonia ... ... | 3·9 |
| Do. ... ... | 2·4 |
| Do. ... ... | 3·7 |
| Chronic bronchitis ... ... | 3·5 |
| Chronic rheumatism ... ... | 4·4 |
| Rheumatism ... ... | 3·5 |
| Mitral regurgitation ... ... | 3·9 |
| Influenza ... ... | 3·7 |
| Do. ... ... | 7·0 |
| Do. ... ... | 7·0 |
| Bright's disease ... ... | 6·5 |
| Pericarditis ... ... | 6·3 |
| Cancer ... ... | 3·4 |

*N.B.*—The amount of the water-precipitable globulin is inversely proportional to the height in inches on the right hand of the table.

## *Conclusions*

(1) The easily precipitable globulins discovered some years ago are responsible for the aldehyde test. They are the same globulins that give rise to the globulin precipitation test and globulin ring test of Brahmachari.

(2) The total content of water-precipitable globulins is generally greater in kala-azar than either in health or in other diseases.

(3) A test has been described here, the *globulin opacity test*, for kala-azar. By this quantitative test a more definite serum test is obtained than any hitherto known.

[*Reprinted from the Calcutta Medical Journal, November, 1923*]

# UREA STIBAMINE IN KALA-AZAR

Proceedings of a meeting held at the Calcutta Medical Club on the 20th September, 1923. Dr. Upendranath Brahmachari read a paper on *Recent Advances in the Antimonial Treatment of Kala-Azar by the use of Urea Stibamine.* Sir Nilratan Sircar presided.

## ABSTRACTS

The speaker said that the first series of his cases treated with urea stibamine appeared in the *Indian Journal of Medical Research* in October, 1922. Major Shortt's paper in the *Indian Medical Gazette* of July, 1923, confirmed his observations as to the leishmanicidal properties of the compound. Subsequent observations of Major Shortt were still more remarkable as three of his recent cases were cured respectively with ·9 grm. in 5 injections, ·75 grm. in 5 injections and ·65 grm. in 4 injections. In each of these cases the cure was established by subsequent observations in hospital and negative results obtained by culture of spleen puncture material. In the present paper Dr. Brahmachari was only limiting his observations on the effect of urea stibamine in the *resistant* or *refractory* cases. By *refractory* or *resistant* cases of kala-azar, he meant cases which had resisted treatment with two grams or more of sodium or potassium antimonyl tartrate given intravenously in the routine form of treatment extending over a period of 2 to 2½ months or more. It was known to every practitioner that a certain percentage of cases was not cured or sometimes not even benefited by antimonyl tartrates unless pushed for a very long time; in some cases, symptoms of intolerance towards the drug would appear after administration of 2 grms. or even less. Some time ago certain observations conducted in Shillong led to the conclusion that all cases of kala-azar were curable with 2 grms. of tartar emetic. Subsequent observations did not confirm this view, as a large majority of cases in Shortt's as well as Mackie's series were not cured with 2 grms. of sodium antimonyl tartrate. In Dr. Brahmachari's series many cases were not cured by 2 grms., but

about 10 per cent of the cases required 5-6 grms. and about 5 per cent were absolutely *refractory*. Some of his cases had 6 grms. and were not cured. He desired every practitioner to keep a record of the failures of the antimonyl tartrates. This would lead to the finding that the last word in the treatment of kala-azar had not been said in tartar emetic or sodium antimonyl tartrate. He observed that this fact as also the very prolonged course of treatment required in most cases justified the demand for further advances in the antimony treatment of kala-azar.

Dr. Brahmachari gave records of about a dozen of *refractory* cases with full details, first describing the effect of treatment with antimonyl tartrates and then reporting the results obtained after intravenous injections of urea stibamine. Short notes of three of these cases are given below :—

(1) Patient R—fever 6 months.

*Condition on admission*—Temp. 99—100° F. Spleen—extended 6 inches below the costal margin.

*Blood*—R.B.C.—3,000,000, W.B.C.—3,500, Hb.—40%. Peripheral blood culture and spleen puncture—positive.

Patient was originally treated with antimonyl tartrates, 6 grms. of sodium and 2·2 grms. of potassium in 75 injections over 6 months. Symptoms of intolerance at times. Had soamin 2 grms. in 11 injections and 6 T.C.C.O., but to no effect. Patient went to Darjeeling and after 2½ months came back almost in the same condition—a *refractory* case.

*Treatment with urea stibamine*—2 grms. in 9 injections starting from ·15, increasing by ·05, given twice weekly. After the third injection fever stopped.

*Condition after the injections*—Fever *nil*; spleen just palpable below the costal margin.

*Blood*—R.B.C.—5,000,000, W.B.C.—6,250, Hb.—70%. Peripheral blood and splenic blood culture and spleen puncture—negative.

*Two and a half months after*—Patient in excellent health, no fever, no enlargement of spleen; blood—normal. Patient cured.

(2) Patient Mrs. L—

*Condition on admission*—Fever 99—100° F. Spleen—extended seven inches below the costal margin. Peripheral blood culture and spleen puncture—positive. R.B.C.—3,000,000, W.B.C.—2,400, Hb.—50%.

Patient was originally treated with potassium antimonyl tartrate—2·8 grms. in 40 injections over a period of 6 months. General condition worse, there was loss of weight with fever of a low intermittent type. Spleen extended 6 inches. Cultural result and spleen puncture—positive. A *refractory* case.

Treatment with urea stibamine commenced one month after the last injection. 1·6 grms. in 9 injections in doses from ·1 to ·25 grm. during 6 weeks.

*Effect of treatment*—One month after completion of treatment—general condition improved. Weight increased by half a stone. No enlargement of spleen. Fever stopped after 5 injections of the salt. Cultural result and spleen puncture—negative.

*Blood condition two months after*—Hb.—60%, R.B.C.—5,000,000, W.B.C.—7,800. Body weight increased by one stone. Patient cured.

(3) Patient N—

*Condition on admission*—Temp. 100—103° F. Spleen extended 5½″ below the costal margin. Blood: Hb.—26%, R.B.C.—2,500,000, W.B.C.—1,000. Peripheral blood culture and spleen puncture—positive. *Cancrum oris* and œdema of the extremities.

Patient was originally treated with sodium antimonyl tartrate—3·8 grms. in 5 injections during 6 months. No improvement of the general condition, no diminution of the size of the spleen, loss of weight by 10 lbs., œdema well marked, *cancrum oris* diminished. Temp. 90-100° F. Symptoms of intolerance after 2·8 grms.

*Blood*—Hb.—32%, R.B.C.—3,250,000, W.B.C.—2,500. Culture and spleen puncture—positive. A *refractory* case.

*Treatment with urea stibamine*—2·85 grms. in 13 injections extending over two months in doses of ·1·to ·25 grm.

*Effect of treatment*—General condition—great improvement. Body weight increased by one stone. No enlargement of spleen. Blood—Hb.—54%, R.B.C.—4,250,000, W.B.C—6,200. Culture and puncture—negative. Patient cured.

The speaker observed that in most of his cases urea stibamine treatment was started about 1½ to 2 months after the stoppage of the previous antimony treatment and as he reported in his paper on the

"*Chemotherapy of Antimonial Compounds in Kala-azar Infection*" in the Indian Journal of Medical Research, that the excretion of antimony was complete by 1 to 1½ months, the question of the effect of residual antimony from the antimonial tartrates in the system did not arise in such cases. As to the purity of the antimonyl tartrates he stated that his salts were chemically pure and were specially made for purposes of research.

All the cases reported were treated with intravenous injections. The speaker stated that both he and Major Shortt were also using the drug intramuscularly. The local reaction was little. But it was not yet time to dwell on the few cases treated intramuscularly though he expected very encouraging results.

He stated that the salt was being prepared in his research laboratory under trained experts and strictest supervision as well as with very strict aseptic precautions. It was repeatedly tested both for toxicity and sterility. The solution was not boiled before use.

Dr. Brahmachari then referred to another compound, stibamine, a name given by him to the sodium salt of *p*-amino-phenyl-stibinic acid. This salt had the same relation to stibacetin as soamin or atoxyl has to ars-acetin. Its chemical and physical properties as also the toxicity had already been published in the *Journal of Tropical Medicine and Hygiene*, August, 1921 and in the *Indian Journal of Medical Research*, October, 1922. He reported three cases treated with stibamine, in two of which the result was satisfactory. The patients were cured with 2·4 and 1·2 grms. respectively. In the third case fever was absent and blood condition improved. Culture and spleen puncture were both negative but the spleen still remained palpable.

Dr. Brahmachari remarked that the duration of treatment and the number of injections required for complete cure with urea stibamine were less than with any other antimony preparations. His results had been confirmed by Major Shortt's observations.

In conclusion the speaker observed that—

(1) Urea stibamine was superior to all other antimony salts for the remarkably short course of treatment. Fewer injections were required for a complete cure.

(2) *Refractory* cases yielded to urea stibamine very strikingly.

(3) No cases had yet been resistant to this salt.

(4) No relapse had yet come to his knowledge, although some of the cured cases were under his observation for nearly two years after complete cure.

(5) There is a possibility of its being used intramuscularly as in the case of atoxyl or soamin.

Dr. Napier after some preliminary remarks gave his experience about urea stibamine which was still very limited. A boy, 12 years old, had been suffering from kala-azar for four months. His spleen was four inches and liver two inches below the costal margin. W.B.C. count was 3,000. He had 10 injections of urea stibamine and at the end of 4 weeks his spleen could not be felt. The important point about the case was that after two weeks his W.B.C. count was over 6,000. Dr. Napier considered the case completely cured. One or two points he would, however, like to speak on the subject of treatment on Dr. Brahmachari's paper. Dr. Brahmachari quoted Major Shortt's experience in Shillong. Personally he (Dr. Napier) was absolutely convinced about the effect of urea stibamine. It was possible that the strikingly good results obtained by Major Shortt with urea stibamine were to some extent due to the climatic condition in Shillong. His experience about the treatment of kala-azar with sodium antimonyl tartrate was that 2 grms. of the salt were not always sufficient in bringing about a complete cure. He observed that in Calcutta very small number of cases were cured by two grams. Dr. Brahmachari's cases could be considered as cured because after the lapse of a year he found that they were in good health. Dr. Napier did not consider that negative results of spleen puncture and blood culture were the last words on the subject of cure. He knew of two cases which gave positive cultural results after a course of treatment with sodium antimonyl tartrate but were subsequently found to have been completely cured after one year without any further treatment.

Dr. Umaprasanna Basu, after referring to his experience in the treatment of kala-azar with sodium antimonyl tartrate in his general practice and in consultation with Sir Leonard Rogers in two of his cases, was of the opinion that sodium antimonyl tartrate was not the specific in kala-azar in the proper sense of the term. In his experience out of 30 or 40 cases only 6 or 7 cases were cured. It took a very long time and then symptoms of intolerance were remarkable. Hence

it could not be a specific and the researches of Dr. Brahmachari had heralded its advent by the discovery of a true specific in urea stibamine. He thought that all were certainly grateful to Dr. Brahmachari for the research he had carried out. The results were very encouraging indeed.

Dr. S. C. Sengupta said that in cases of kala-azar the impression that one forms of sodium antimonyl tartrate was that a large number of cases did very well but every case took a very long time. On the other hand in many cases the spleen did not diminish in size, and R.B.C. as well as W.B.C. as also Hb. did not seem to increase. Then some of these cases showed intolerance to the drug and some developed broncho-pneumonia, dysentery or diarrhœa during treatment with sodium antimonyl tartrate. He had observed that a certain number of cases which did not do well with sodium antimonyl tartrate, subsequently seemed to do well when potassium antimonyl tartrate was used. Dr. Brahmachari has stated elsewhere, that cases which did not do well with sodium or potassium antimonyl tartrate treatment did well with metallic antimony. His own impression was that antimony was really the specific for kala-azar. But as to the salt which should be used, of course we could not do better than to leave the question to Dr. Brahmachari to solve. He did not know the reason why a particular case did very well up to a certain limit and why it was that it did not do well any further. His criticism was only an enquiry whether they should be satisfied with urea stibamine as a salt pre-eminently specific for kala-azar. He asked whether the variable solubility of a particular antimony salt got anything to do with the destruction of the parasite.

Dr. J. M. Das said that he had seen that sodium antimonyl tartrate in some cases was quite useless. Sometimes actual danger was apprehended. He observed that if in any way the W.B.C. could be increased then antimonyl tartrates did well. He was trying in his own way to increase W.B.C. and he got very good results. He gave antimony and tried to increase the leucocytes, by bringing on an artificial inflammation by injecting several substances along with sodium antimonyl tartrate. He suggested different things to the mofussil practitioners including the stem of *nim* as "*gool*" under the skin and into the muscle. While sodium antimonyl tartrate absolutely failed,

this process generally brought on good results. In his experience where sodium antimonyl tartrate failed, potassium antimonyl tartrate did well. Hence before they passed any remarks against the antimonyl tartrates they should consider them more leniently and from a wider point of view.

Dr. Satya Saran Mitra said that from the observations made by Dr. Brahmachari he noticed that the swing of the pendulum had been turned against tartar emetic and sodium antimonyl tartrate and some other antimonial compound was making a rapid headway pushing them to the background. People were getting accustomed to see only one side of the thing and they thought that sodium antimonyl tartrate was really the panacea although it was sometimes producing very disastrous results. Now that some kind of check was put on the swing of the pendulum, people would be on their guard in the use of antimonyl tartrates. He asked Dr. Brahmachari to express his opinion and observations on the use of the new drug urea stibamine by the intramuscular route and whether he found it to be efficacious in the treatment of many diseases which were as fell as kala-azar, such as the infantile cirrhosis of the liver. He wanted to know the circumstances that led Dr. Brahmachari to the discovery of the new drug.

Dr. Bepin Behari Gupta said that this lecture was heard by him in the Asiatic Society of Bengal. It was glorious that a Bengalee made this most important advancement in the treatment of a disease which was really black, black in its treatment, black in all its aspects and that blackness was going to be whitened by one of Bengal's own men.

### Dr. Brahmachari's Reply

He discussed the various points raised by the gentlemen present and said :

"First of all I must thank Dr. Napier most heartily for his remarks. I do not think that the good results obtained by Major Shortt in Shillong were to any great extent attributable to good climate. If we go through his last paper that was published in the *Indian Medical Gazette* we will find that there were resistant cases, cases which were resistant to the antimonyl tartrates even in Shillong inspite of the best climate possible there. Of course climate, to some extent, may have some influence but there was something more in urea stibamine that was responsible for the cure of cases of kala-azar in such a remarkable way as recorded by Major Shortt.

"As regards complete cure, I had the patience of observing a series of cases kept in the Campbell Hospital for about 2 years for observation. The patients are still there and are perfectly cured. There is no enlargement of the spleen, blood count is normal and the culture from spleen puncture as well as from peripheral blood is negative. There does not exist any sign of the disease in these patients. Of course the definition of cure of a disease must be very difficult. If, however,one or two years have passed and no symptoms and no trace of the disease have been found, the blood count is quite normal, there is no anæmia and the blood culture and spleen puncture are negative, I do not think one should question their cure. Although we cannot possibly claim that the last word about the treatment of kala-azar has been said in urea stibamine, I hope that the discovery of this salt has advanced greatly the treatment of the disease. At least we can say that the antimonyl tartrates were not the last words. There is no doubt that research must be carried on along proper lines so that we can bring out, for the treatment of kala-azar, specifics like salvarsan or neo-salvarsan. Urea stibamine being allied to soamin or atoxyl is a step towards the discovery of such compounds. From the study of atoxyl I felt that the corresponding urea salt of antimony might be very suitable for the treatment of kala-azar. It also struck me that being an urea ester, it would be more suitable for intramuscular injection as urea is a local anæsthetic. This was the origin of urea stibamine and my speculations have been justified by actual experience. There is no doubt that the cases read before the meeting this evening distinctly prove that we have made a very great advance in the treatment of kala-azar by the use of urea stibamine.

"It cannot be said that insufficient doses of the sodium or potassium antimonyl tartrate were always responsible for making cases *refractory*. My cases were really very resistant and I think you will agree with me in this point.

"As regards the value of intramuscular injection of urea stibamine I cannot say anything definitely till after some more experience with the drug. I am, however, glad to say that I met with success in two cases in which urea stibamine was used intramuscularly without any local reaction or pain. But more cases must be studied before we come to a definite conclusion about the intramuscular use of urea stibamine.

"As regards the advantage of alternating sodium and

potassium antimonyl tartrates, I hold that it really exists in some cases, but in the refractory cases described in my paper this evening this was not so.

"As regard Dr. Mitra's remarks that the swing of the pendulum has gone back the other way, I agree with him that the swing has gone back so far as the antimonyl tartrates are concerned but otherwise it has got a fresh momentum and the swing is always going forward. Many of us once thought that sodium antimonyl tartrate was the specific in every case of kala-azar but it is not so.

"As regards the supply of urea stibamine it is certainly limited. I prepare urea stibamine in small quantities and I am giving it a trial in a scientific way. I cannot say whether I can at present supply larger quantities.

"It now remains for me to express my thanks to the Indian Research Fund Association for their valuable assistance in carrying on my researches."

## PRESIDENT'S REMARKS

"I think I voice the sense of the whole house when I say that we offer our heartiest congratulations to Dr. Brahmachari on this occasion. Ours are double congratulations. One is for the discovery of this urea antimony salt and the other for the application of that salt to the treatment of kala-azar.

It is scepticism that is the basis of all scientific progress in this world. It simply means that whenever any evidence is put before us it should be properly examined and then accepted, for unless it is so done, it is likely to crumble in the long run. Well, applying this principle in this particular case, it is our duty to gather all information in regard to this matter with which we are so deeply concerned, I mean, the treatment of kala-azar. Dr. Brahmachari adduces evidence of the strongest character that so far urea stibamine is the best agent for the treatment of kala-azar in his and Major Shortt's hands. Well, we accept it so far as is concerned to himself or Major Shortt. But to say that it has very greatly advanced the treatment of kala-azar, I would take a little time, and I think Dr. Brahmachari would not grudge me the time I want, because if after a couple of years we say that it is a great advancement in the treatment of kala-azar, I think that word will be really and truly

said. My suggestion is this, that a very good *prima facie* case has been made out on the evidences put forward by Dr. Brahmachari's experience as well as that of Major Shortt in Assam. So far as they go they are indisputable. Nevertheless we think these findings may have limitations. A few years ago, it must be admitted, great expectations were entertained about the treatment of this fell disease with sodium antimonyl tartrate. I do not think that hope has crumbled into dust to-day. But every case cannot be expected to be successful. In course of time some of our apprehensions have been realised. We hope we should not have a similar experience in regard to this new salt. It is possible that in the course of a few years our indefatigable worker will have again to draw our congratulations upon the discovery of yet a better and more efficient drug for the treatment of this disease. I think that considered from the chemical, physiological and pharmacological points of view so far urea stibamine has stood the test in his own and Major Shortt's hands and I believe, it will stand the test with many of us.

"Whatever the future may prove, even if we find out some improvement in the treatment of kala-azar, we shall always remain proud of Dr. Brahmachari. At this moment this salt comes as a real friend to enable us to help many of our brothers and sisters to be cured of this fell disease, and we can surely realise how great is our delight to be able to depend upon one of our home-made products for the purpose of vanquishing one of our worst enemies. No German would have stronger reasons of being proud of the Krupp gun nor a Frenchman would have greater reasons of being proud of his navy than we Bengalees would have, being able to use one of our home-made products for the purpose of fighting out one of the most dreadful diseases through the discovery of our Dr. Brahmachari. We have every reason of being proud of what has come out of Dr. Brahmachari and I believe that in the future we shall have occasion to hear from him fuller accounts about his work in the treatment of this fell disease."

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# THE RELATION BETWEEN THE CHEMICAL CONSTITUTION OF ANTIMONIALS AND THEIR THERAPEUTIC PROPERTIES

In this paper the relationship of the chemical constitution of the following antimonials to their therapeutic properties are discussed :—

(1) Metallic antimony.
(2) Antimony trioxide.
(3) The antimonyl tartrates.
(4) Aromatic antimonials—urea stibamine.

Antimony belongs to the odd series of Group V of Mendeléeff's periodic system of elements in which the gradual transition from typical non-metals to typical metals is clearly exhibited. Phosphorus is decidedly a non-metal, while antimony and bismuth are typical metals, although they are brittle. Arsenic, which stands between these two classes, shows properties belonging to both groups of elements. The acid-producing properties of antimony are greater than those of bismuth and less than those of arsenic. It has the property of combining with tartaric acid and giving rise to an acid of the type of what has been termed antimonyl tartaric acid. Tartar emetic and sodium antimonyl tartrate should not be regarded as antimony salts of an organic acid. They are really potassium and sodium salts of antimonyl tartaric acid as has been proved by Clarke, Stallo, Jungfleisch, Guntz, Adam and others. Antimony exists in them not as a basic $-Sb=O$, in combination with

tartaric acid, but as ortho-antimonious acid, $Sb(OH)_3$, in which two of its hydroxyl groups are replaced by the divalent group $C_4H_4O_6$ giving rise to antimonyl tartaric acid. In other words, antimony in tartar emetic and allied compounds exists in an acidic state.

The most important factor upon which the therapeutic value of an antimonial depends, is its property of containing trivalent antimony in an acidic state or its ability of being converted into a compound of this kind after its introduction into the body which will further possess the mobility of being converted into a compound containing the radicle $-Sb{=}O$ in a reactive state or in a highly dispersed condition.

The *reactive* state corresponds more or less to the *nascent* state of elements and *mobility* means the quickness with which the property referred to above is displayed.

The antimonyl tartrates, finely divided metallic antimony or an aromatic antimonial derived from stibanilic acid, more or less possess these qualities and the superiority of an antimonial over another depends upon the degree of its power of exhibiting them.

In studying trypanosomiasis, Ehrlich held the view that trypanosomes assimilated the organic derivatives of arsenic only when the arsenic was present in the trivalent and not in the pentavalent form. Similarly the experiments of Kolle, Hartoch, Rothermundt and Schürmann have shown that compounds containing pentavalent antimony were not organotropic except in large doses and were, at the same time, slightly parasitotropic. Preparations containing trivalent antimony were, as a rule, toxic to the organism and at the same time it was also shown by these observers that for antimony compounds, soluble or insoluble, organic or inorganic, to be of therapeutic value in trypanosomiasis, the antimony must be in the trivalent form. My most recent researches and those of others that have followed

me have, however, proved that the aromatic pentavalent antimonials are much more potent in the treatment of kala-azar than the trivalent antimonyl tartrates. I hold that it is not so much whether an antimony compound is trivalent or pentavalent that is responsible for its therapeutic value, but its capacity for being quickly converted into a compound containing $-Sb=O$.

By studying the excretion of antimony in man after intravenous injection of the aromatic antimonials of the type of urea stibamine and also of the antimonyl tartrates, one can explain the superiority of the former over the latter on the above theory. I have observed that in the case of tartar emetic, the curve of excretion is one slowly converging to the base line.

The amount of antimony excreted in the urine during the first 24 hours after intravenous injections of tartar emetic is about 6 per cent of the amount injected. The amount of antimony excreted in the urine during the first 24 hours after intravenous injections of urea stibamine is 30 to 40 per cent of the amount injected. The excretion of antimony after intravenous injections of a pentavalent organic antimonial follows a curve, the first portion of which, representing the excretion during the first 24 hours, is abrupt, and the second portion follows a course similar to that found in the case of tartar emetic. It is probable that a pentavalent organic antimonial is converted in the body into a trivalent antimonial and that as long as it exists in the body in the pentavalent form, its rate of excretion is much quicker than when it is converted into the trivalent form. During the latter stage the curve of excretion is similar to that of tartar emetic in which antimony exists in the trivalent form. Since a great portion of antimony present in an aromatic pentavalent antimonial (urea stibamine) is quickly eliminated, the chances of toxic action of the compound are much less than that of an antimonyl

tartrate. In the process of conversion of an aromatic pentavalent antimonial in the body into a compound containing trivalent antimony, a reactive $-Sb=O$, is formed, which is probably responsible for the remarkably beneficial results observed in the treatment of leishmaniasis by the use of urea stibamine.

### *Finely Divided Metallic Antimony*

Though various metals have been administered intravenously in the colloidal state, metallic antimony is perhaps the only one which has been put into the circulation in the crude form of a fine suspension. To Plimmer and Fry belongs the credit of first demonstrating the possibility of introducing metallic antimony into the veins without danger of capillary blocking. To Ranken belongs the credit of using the drug successfully in man in the treatment of trypanosomiasis by the intravenous route.

In 1915 I described a number of cases of kala-azar treated successfully with intravenous injections of metallic antimony and I pointed out that it was the most powerful leishmanicide that was known at that time, just as it was the most powerful of the known antimonial trypanocides.

I observed that in cases in which the soluble salts of the type of tartar emetic did not show any improvement in the blood condition or temperature of the patient after several injections, finely divided metallic antimony administered intravenously brought about complete cure. In addition, the number of injections required for a course of treatment with metallic antimony was much smaller than those required in the case of the antimonyl tartrates. Three or four injections frequently cured the patient, though sometimes the injections required were as many as eight or nine. Even then the number of injections required for cure was less than what was generally required in the

case of antimonyl tartrates. The only objection is the complicated technique of the operation of injection which is a serious obstacle to mass treatment of the disease.

The mechanism by which metallic antimony is taken up into the system after intravenous injections is very interesting. Quickly taken up by the leucocytes, and perhaps also by the cells of the reticulo-endothelial system and without causing any capillary blocking, it is converted into a soluble antimony compound, as the particles of antimony sooner or later disappear from the leucocytes. I consider that it is converted into a compound in which the antimony exists in a trivalent state and this conclusion has been arrived at by me by following the curve of excretion of antimony in the urine after its administration which resembles the curve of excretion of antimony after administration of tartar emetic.

This trivalent antimony compound is subsequently converted into one containing a radicle of $-Sb=O$ in the reactive stage or in a highly dispersed condition. It does not rest at the stage of a trivalent antimony compound allied to $Sb_2O_3$, because $Sb_2O_3$, when injected intravenously, does not exhibit such therapeutic properties as those of metallic antimony, as will be presently seen. It is possible that it is finally converted into nascent metallic antimony.

Levaditi has propounded a general law with reference to all the members of the nitrogen family of elements, occupying Group V of Mendeléeff's periodic table such as arsenic, antimony or bismuth. They or their compounds exhibit their parasiticidal properties only after they have been acted upon by the tissues. If fresh extract of liver is added to them, then they exert their parasiticidal properties.

I have observed that if a solution of tartar emetic or urea stibamine is mixed with a culture of the flagellated form of leishmania and the mixture examined under the microscope, they do not die. Like bismuth or its compounds,

they become active only after they have been acted upon by the tissues.

It has been suggested that in the case of bismuth, the action of the cellular extract gives rise to a new compound, '*bismoxyl*,' and it is this which possesses the destructive power against the *Treponema pallidum*. The substance in the extract which has the property of changing bismuth into bismoxyl, has been termed '*bismogene*.'

Bismoxyl is supposed to be a bismuth toxalbumin.

Chemically, some of the bismuth compounds contain the radicle $-Bi=O$, just as some of the antimony compounds contain the radicle $-Sb=O$, and it is very likely that the bismuth toxalbumin also contains the radicle, $-Bi=O$, in the reactive stage, or in a highly dispersed condition. A corresponding antimony compound, which may be called '*stiboxyl*, is probably formed in the case of antimony.

It has been recently observed by Meleney that in kala-azar, clasmatocyte tissue is developed as a tissue reaction and probably, as I have suggested, out of the reticulo-endothelial system. I hold that this reticulo-endothelial system gives rise to the production of bismoxyl or stiboxyl as the case may be.

I have discussed in detail what, I consider, is the mechanism by which metallic antimony exerts its parasiticidal properties, because, being a simple element, it does not contain any groups or radicles which may complicate any explanation that may be suggested. The sequence of events in this mechanism may be summarized as follows :—

Metallic antimony—taken up by leucocytes and cells of the reticulo-endothelial system—a soluble trivalent antimony compound—an antimony compound containing $-Sb=O$ in the reactive stage or dispersed condition (stiboxyl), or nascent antimony which acts as a leishmanicide in kala-azar.

### *Colloidal Metallic Antimony*

Colloidal metals are remarkable in having minimum organotropic properties and at the same time are frequently

parasitotropic. An ideal medicament should be one in which the ratio of *dosis curativa* to *dosis tolerata* should be as low as possible. Because of the extreme division of the metals in the colloidal state, there is an immense surface of contact between a colloidal solution or suspension and the surrounding medium. For instance, it has been calculated that the total surface of particles of gold in one cubic centimetre of colloidal gold may attain to nearly 6,500 square feet. This immense contact surface of the colloidal suspension of metals, their electric charge of constant sign for the same substance and the fact that in the living body the reactions that take place are nearly always between colloids, render the potentialities of metallic colloids very great.

### *$Sb_2O_3$—Antimony Trioxide*

Yorke and Blackmore have used trixidine in oily suspension intramuscularly. They have also used a fine preparation of the same intravenously.

Kolle, Hartoch, Rothermundt and Schürmann consider that the formation of a deposit of an insoluble slowly absorbable compound of antimony, such as antimony trioxide, acts prophylactically against trypanosome infection. The principle of the employment of insoluble organic compounds of antimony, either in ointment form or through the formation of intramuscular depôts, constitutes what the authors designate *therapia mite curans*, as contrasted with *therapia magna sterilans*. Rogers used it in kala-azar. In my experience it is weak in its therapeutic properties in kala-azar.

I shall now try to explain why antimony trioxide is feebler in its leishmanicidal properties than metallic antimony. Though antimony exists in it in the trivalent state, yet its proneness for being converted into a compound containing $-Sb=O$ in a reactive state is slight, as it is a fairly stable compound. This theory agrees with the fact that it is

more potent than $Sb_2O_5$ which is more stable and has much less leishmanicidal properties. Of all the oxides of antimony $Sb_2O_4$ is the most stable. So far as I am aware, $Sb_2O_4$ has no use whatever in therapeutics. One may, therefore, lay down as a general rule that the more stable an oxide of antimony is, the less is its trypanocidal or leishmanicidal property.

### *Tartar Emetic and other Antimonyl Tartrates*

As stated before, these are salts of antimonyl tartaric acid and have been erroneously considered as antimony compounds of tartaric acid even in a recent textbook on kala-azar.

Among the antimonyl tartrates of the type of tartar emetic or sodium antimonyl tartrate may be mentioned ammonium antimonyl tartrate, urea antimonyl tartrate, aniline antimonyl tartrate, ethyl antimonyl tartrate, quinine antimonyl tartrate, cinchonine tartrate and narcotine antimonyl tartrate.

If T $(NH_4)$, T (Urea), T (K), T (Na), T (Aniline), etc , represent the toxicity of the above tartrates respectively, I have observed :—

$$\frac{T\ (NH_4)}{T\ (Urea)} = \frac{T\ (NH_4)}{T\ (K)} = \frac{T\ (NH_4)}{T\ (Na)} = \frac{T\ (NH_4)}{T\ (Aniline)} = \frac{55}{60} \text{ or } \frac{11}{12}$$

If T. Sb $(NH_4)$, T, Sb (Urea), T. Sb (K), T. Sb (Aniline), T. Sb (Na) represent the toxicity of the antimony content of the above tartrates, we have :—

$$\frac{T.\ Sb(NH_4)}{T.\ Sb(Urea)} = \frac{41}{46};\quad \frac{T.\ Sb(NH_4)}{T.\ Sb(K)} = \frac{40}{46};\quad \frac{T.\ Sb(NH_4)}{T.\ Sb(Na)} = \frac{38}{46};\quad \frac{T.\ Sb(NH_4)}{T.\ Sb(Aniline)} = \frac{35}{46}.$$

Therefore, in the case of the guinea-pigs, ammonium antimonyl tartrate is the least toxic, then comes the urea salt, then the sodium and potassium salts which are equally toxic and then the aniline salt.

The maximum tolerating capacity of the same species of animals for a drug is *directly proportional* to its maximum tolerated dose.

We thus have :—

(1) Maximum tolerating capacity of guinea-pigs treated with antimonyl tartrate $= K^1 \times 03$

(2) Do. Do. Do. Urea antimonyl tartrate $= K^1 \times \cdot 025$

(3) Do. Do. Do. Potassium antimonyl tartrate $= K^1 \times \cdot 015$

(4) Do. Do. Do Sodium antimonyl tartrate $= K^1 \times \cdot 015$

(5) Do. Do. Do. Aniline antimonyl tartrate $= K^1 \times \cdot 025$.

From this we conclude that of all the antimonyl tartrates used in the case of the guinea-pigs, their maximum tolerating capacity is with ammonium antimonyl tartrate and that the presence of N in the basic radicle of an antimonyl tartrate diminishes the toxicity of some of them.

Generally speaking, the toxicity of the antimonyl tartrates depends upon their antimony content. A notable exception is in the case of quinine antimonyl tartrate in which the toxicity is low. The possibility of using the compound in therapeutics should therefore be borne in mind, as it may combine the therapeutic properties of antimony and quinine.

I have not, however, been able to confirm the observations of Farghar and Gray that the toxicity of the antimony content of quinine antimonyl tartrate is only one-fifth that of tartar emetic, though I agree with them that its toxicity is less than that of tartar emetic. I confirm their observations that quinine antimonyl tartrate, on boiling with antimony trioxide, is converted into the more toxic quino-toxine antimonyl tartrate. I have not been able to confirm their conclusions that the sodium salt is less toxic than the potassium salt. I have confirmed Plimmer and Thompson's

observations that the lithium salt is more toxic than the sodium or potassium salt and that the toxicity of the sodium and potassium salts is equal.

I have further found that ammonium antimonyl tartrate is the least toxic of all the inorganic tartrates, the presence of nitrogen in the basic radicle diminishing its toxicity. Because of the high antimony content of the ammonium salt, its relatively low toxicity for the lower animals and likewise for human beings, and since it was found to possess a marked degree of therapeutic activity in the treatment of kala-azar, I consider it superior to both potassium and sodium antimonyl tartrates.

I found that after the administration of a toxic dose of an antimonyl tartrate, the pathological changes are most marked in the lungs, kidneys, liver, pituitary and suprarenal glands, consisting chiefly of hæmorrhages into the substance of these organs and destruction of their cellular elements. Similar changes were produced by toxic doses of the new aromatic organic antimonials.

*Delayed Antimony Poisoning.*—Cases of death in guinea-pigs three weeks or so after one injection of an antimonial salt have been met with, showing definite symptoms of antimony poisoning and presence of antimony in the viscera.

These cases of delayed antimony poisoning are of very great clinical importance, as they prove that the excretion of the drug may sometimes be very slow after injection of antimonial compounds and some of the cases of sudden death during antimonial treatment may be due to a cumulative action of the drug.

*Cumulative and Tolerance Experiments with Tartar Emetic.*—I have observed that repeated injections of tartar emetic in sub-lethal doses did not give rise to any tolerance towards the drug except very rarely. Generally the results pointed to a cumulative action of the drug, or at least made the animal susceptible to the next higher dose.

I have observed before that antimony in tartar emetic and other antimonyl tartrates exists in the form of antimonious acid, $Sb(OH)_3$, in which two hydroxyl groups have been replaced by the divalent $C_4H_4O_6$. When introduced into the system, its therapeutic value depends upon its ability to give rise to a reactive $-Sb=O$ which, theoretically speaking, should be the same as that of the salts of hydrated $Sb_2O_3$, *i.e.*, $Sb_2O_3+3H_2O$, or $Sb(OH)_3$, or ortho-antimonious acid. Herein lies the superiority of the aromatic antimonials over the antimonyl tartrates which we shall presently see. On the other hand, if it were possible to prepare an antimonial having the same composition as the antimonyl tartrates but having the radicle $-Sb=O$, as is shown in the old configuration of tartar emetic and allied salts, then such an isomer of tartar emetic would be more potent in the treatment of kala-azar than tartar emetic itself. We await the production of such an isomer.

Besides the antimonyl tartrates already referred to, the following amino-antimonyl tartrates have been prepared in my laboratory :—

(1) Phenocoll antimonyl tartrate.
(2) Anæsthesin antimonyl tartrate.
(3) Novocaine antimonyl tartrate.
(4) Aposthesine antimonyl tartrate.
(5) Orthoform antimonyl tartrate.
(6) Acriflavine antimonyl tartrate.

The late Sir Patrick Manson once wrote to me as follows: "Go on in your efforts to get an antimony compound that can be used as an intramuscular injection or, better still, as a drug that can be administered by the mouth."

The therapeutic value of an antimonial depends upon its concentration in the tissues after administration.

Unfortunately ordinary antimonials cannot be administered orally, intramuscularly or per rectum in such doses as to bring about this concentration without at the same time giving rise to local distressing symptoms. The above new amino-antimonyl tartrates containing radicles, possessing anaesthetic properties, may be worth trial by these routes.

Ointment of metallic antimony in a state of finest subdivision may be more easily absorbed and less irritating than that made with ordinary metallic antimony, and may be of therapeutic value in the treatment of kala-azar.

### *Aromatic Antimonials*

Let us now pass on to the consideration of the aromatic antimonials and their value in the chemotherapy of antimony.

In 1920, shortly after I had been financed by the Indian Research Fund Association for carrying on researches into the treatment of kala-azar, I brought to the notice of the Government and the Governing Body of the Indian Research Fund Association the possibility of the potentialities of organic antimonials in the treatment of Indian kala-azar, my conclusions being based on theoretical grounds, from an analogy of the value of the corresponding compounds of arsenic, namely, *ars-acetin* and *atoxyl*, in the treatment of certain protozoal diseases.

The acetyl compound (*stibacetin*, *stibenyl*) was used more or less successfully outside India in the treatment of kala-azar and other forms of leishmaniasis (Caronia, Kharina-Marinuchi, Spagnolio). Manson-Bahr successfully used it in a case of kala-azar. Early in 1921, I discovered that urea could combine with stibanilic acid and that the resulting compound surpassed all my expectations in its value in the treatment of kala-azar. The discovery of this compound and my researches into the chemotherapy of antimonial com-

pounds in kala-azar infection opened up a new vista in the treatment of the disease.

The starting material of aromatic antimonials is acetyl-*p*-amino-phenyl-stibinic acid. Theoretically speaking, the value of the sodium salt of the acid in the treatment of kala-azar should be the same as that of ars-acetin in the treatment of trypanosome infection. Ars-acetin has certain marked advantages compared with atoxyl, being more stable and less toxic to some animals, while equally toxic to the parasites. This diminution of toxic effect is, however, noticeable only in certain animal species and not with horses or guinea-pigs. Voegtlin and Smith have observed that it is considerably less toxic than atoxyl and more trypanocidal, possessing a chemo-therapeutic index about five times higher than atoxyl.

It is a well-known theory in the case of aromatic arsenicals that their therapeutic value depends upon the reduction products produced after their introduction into the system. These reduction products probably all contain the reactive $-As=O$. The trivalent aromatic arsenicals of the arseno-benzene group possess the property of producing these reduction products to a greater extent than the pentavalent arsenicals and hence their superiority in the treatment of treponema and trypanosome infections over the pentavalent arsenicals except tryparsamide. I hold that the therapeutic value of the aromatic antimonials also depends upon the same property.

The comparative value of the aromatic antimonials in the treatment of kala-azar also depends upon their toxicity and parasitotropic properties following their administration. These, again, depend upon their chemical configuration and physico-chemical properties. In order that they may be of therapeutic use the ratio of their *dosis curativa* to that of their *dosis tolerata* must conform to Ehrlich's formula, which is 1 : 3 or less.

### *Aromatic Antimonials of the Stibino-Benzene Group*

Antimonials of the stibino-benzene type have not yet come into use in the treatment of human diseases, though they have been used with indefinite results in the case of certain diseases of animals.

Trivalent aromatic antimonials of the type of salvarsan or neo-salvarsan will probably be in future the highest advance in the antimony treatment of kala-azar.

### *Aromatic Antimonials derived from p-Stibanilic Acid (p-Amino-phenyl-stibinic acid)*

### *Acetyl-para-aminophenyl-stibinate of sodium (Stibenyl, Stibacetin, Sodium acetyl-p-stibanilate)*

The minimum lethal dose of phenyl stibinate of sodium, is three and half times less than that of acetyl-*p*-amino-phenyl-stibinate of sodium, while its maximum tolerated dose is 35 times less. Injected into lower animals, it gives rise to hæmorrhagic nephritis and other symptoms of severe antimony poisoning. This compound has little or no use in therapeutics, but the introduction of $NH_2$ into its benzene nucleus at once diminishes its toxicity and raises its therapeutic value to a remarkable extent.

The acetyl compound of antimony has been used in the treatment of kala-azar but with unsatisfactory results. Besides, as has been shown by me, stibenyl becomes toxic with age in India and it has now come into disuse. But I still hold that pure acetyl-*p*-amino-phenyl-stibinate of sodium should again be given a trial in kala-azar and may in future be found to be free from all those toxic effects that were exhibited by stibenyl.

The sodium salt formed after hydrolysis of the acetyl compound corresponds to atoxyl or soamin and is sodium-*p*-stibanilate. Comparing its toxicity with that of the acetyl

compound, it will be seen that the introduction of the acetyl group into it does not reduce its toxicity as in the case of the corresponding arsenic compound. Thus, while, in the case of ars-acetin, the toxicity is markedly diminished by the introduction of the acetyl group into atoxyl, being $\frac{1}{5}$ that of atoxyl in the case of sodium stibanilate and the acetyl compound, my observations have shown that their toxicity is the same. The M.L.D. is 0·7 grm. per kilo of body-weight and the M.T.D. is 0·35 grm. per kilo of body-weight in guinea-pigs given intramuscularly in the case of both the compounds.

The pure salt is fairly stable. It has been stated by some observers in India that the compound is very easily decomposed. Evidently, the substance that they were using was impure or not properly prepared. Three cases have been treated by me with this compound with satisfactory results. But as the number of cases was limited, no attempt can at present be made to give a comparative estimate of the therapeutic values of sodium-*p*-stibanilate and urea stibamine—a compound to be discussed later on.

*Chloro Stibacetin (von Heyden '471') or Stibosan*

This is a compound formed by the replacement of one hydrogen atom in the benzene nucleus of the acetyl compound by chlorine. The published results of cases treated with this compound lead to the conclusion that it is weaker in its therapeutic effects when compared with urea stibamine. It has been claimed that the introduction of chlorine increases its stability. It has also been claimed that it can be stored in ordinary stoppered bottles and weighed out when required and is therefore most useful for general purposes. In my opinion such a compound has more or less the same stability as the antimonates and therefore there is less chance of the production of the reactive $-Sb{=}O$ in the tissues after

their administration which, I consider, is responsible for the beneficial results following the administration of an antimony compound. This explains why antimonates in which antimony exists in a pentavalent form are of very little use in therapeutics, as they are very stable and quickly excreted unchanged after administration. The same also holds good in the case of arsenic.

### *Urea Stibamine*

The next aromatic antimonial discovered by me is urea stibamine. I shall discuss its therapeutic value later on.

### *Benzene-sulphon-p-amino-phenyl-stibinate of Sodium*

The next aromatic antimonial of probable therapeutic value that has been discovered by me is benzene-sulphon-*p*-amino-phenyl-stibinate of sodium. The corresponding arsenic compound is known as hectine which possesses certain therapeutic properties in the treatment of syphilis. The entrance, however, of a sulphonic group in the molecule reducing its toxicity also reduces its therapeutic properties and this fact is in accordance with the general physiological inertia of the sulphonic acids.

### *Sodium-allyl-thiocarbamino-p-stibanilate*

Sodium allyl-thiocarbamino-*p*-stibanilate is another compound of probable therapeutic value which has been produced in my laboratory. The introduction of thio-urea may reduce the toxicity of the compound, just as it has been claimed in the case of the corresponding arsenic compound.

### *Glucose Derivatives*

The therapeutic value of the glucose compounds of the organic aromatic antimonials, as compared with compounds

from which they are derived, is proportional to their antimony content and the same conclusion is arrived at on theoretical considerations. Their antimony contents are less than the corresponding aryl-antimonials from which they are derived and therefore a bigger dose has to be administered to be of equal therapeutic value. The combination with glucose has therefore no advantage.

The antimony content of some of the aromatic antimonials is given below :—

| | |
|---|---|
| Sodium stibanilate ... ... | 42·10 per cent |
| Urea stibamine ... ... | 36·95 ,, |
| Chloro-stibacetin (Stibosan) ... ... | 33·30 ,, |
| Glucose sodium stibanilate ... ... | 25·80 ,, |
| Glucose urea stibamine ... ... | 23·80 ,, |
| N-phenyl-glycine-amide-*p*-stibinate of sodium | 29·30 ,, |
| Neo-stibosan ... | 42·0 ,, |

It may be stated that, generally speaking, the therapeutic value of the aromatic antimonials derived from *p*-amino-phenyl-stibinic acid is proportional to their antimony content.

As regards toxicity, I have observed that the toxicity of pentavalent compounds obtained from *p*-stibanilic acid is proportional to their antimony content. My observations are different from what I find stated in a recent book on kala-azar, from which it will be seen that urea stibamine and its glucose derivatives are regarded to be equally toxic. The latter observation is rather significant as this would mean that the antimony content of the former is one and a half times less toxic than its glucose derivative.

It is a well-known fact that the sodium salt of N-phenyl-*p*-arsenic acid is a substance of practically no importance in

the treatment of experimental infections such as those produced in laboratory animals by various species of trypanosomes, the spirochætes of relapsing fever and *Treponema-pallidum*. On the other hand, N-phenyl-glycine-amide-*p*-arsenate of sodium, which is known under the trade name of *tryparsamide*, is the most effective arsenical yet produced for the treatment of human sleeping sickness. It has been stated that as Ehrlich's reduction theory proved fruitful in producing results of highly practical value, it does not represent the whole truth, for in tryparsamide, arsenic exists in the pentavalent form. But I hold that if a pentavalent organic arsenical, when introduced in the system, is more quickly converted into a compound containing the reactive $-As=O$ than arseno-benzene compounds, then the former will be of more therapeutic value than the latter and on this the value of tryparsamide can be explained on Ehrlich's reduction theory.

### *Urea Stibamine*

The most important and the last antimony compound that I shall now discuss is urea stibamine. It is somewhat allied to tryparsamide.

These compounds contain the group $NH_2CO$ and, this, to my mind, is responsible for the therapeutic value of tryparsamide. That being the case, one would expect to have the same remarkably beneficial effects with antimony compounds containing this group in the treatment of diseases in which antimony is indicated, just as tryparsamide in the case of human trypanosomiasis.

This theoretical conclusion is borne out in practical experience. For to-day urea stibamine stands as the most pre-eminent compound of antimony in the treatment of kala-azar.

Taking into consideration the published kala-azar cases of different observers under different conditions and in

different places treated with the aromatic antimonials, the most extended trial has been given to urea stibamine. Observations upon the other aromatic antimonials are mostly limited to observations of single individuals.

In a combined series of 325 published cases which were treated by myself, Shortt, Greig, Kundu and others with this compound, 98·47 per cent. of the cases were cured. One of the cases died of extreme asthenia, being admitted at the age of 65 in a moribund condition. In 298 of these cases, proof of cure was microscopic and cultural examinations and disappearance of symptoms and in 27 cases, proof of cure was clinical disappearance of the symptoms and subsequent observations of the cases. One case was resistant.

Regarding the value of N-phenyl-glycine-amide-*p*-stibinate of sodium in the treatment of kala-azar, I have published a series of eight cases in which it was successfully used but no comparison can be made at present with urea stibamine as it has not yet been given an extended trial in the treatment of the disease.

It has been proved by the observations of Shortt as well as those of myself and others that urea stibamine does not manifest any deterioration or other changes either in physical and chemical characters or in therapeutic properties, if kept in sealed ampoules under ordinary conditions. A more stable compound is undesirable as it will be less effective.

I have already referred to my views of the reticulo-endothelial system and I hold that individual cases will get beneficial results from the use of antimony compounds proportional to the reaction of the reticulo-endothelial system. Two things are necessary, namely, the development of the clasmatocytes and introduction of an antimony compound with which they can combine for the development of stiboxyl. Herein lies the value of the different antimonials and the superiority of urea stibamine over the other antimony

compounds. This also explains why, with the same antimony compound, one individual is cured much more quickly than another after its administration. It is the response of the cells of the reticulo-endothelial system to a particular drug that one should aim at in the treatment of the resistant cases.

Let me now briefly refer to the views of Voegtlin and his co-workers. These observers have pointed out that arsenious oxide and its derivatives combine with substances containing a sulphydrile grouping and that the toxic action of the organic arsenoxides is depressed by the simultaneous injection of excess of sulphydrile compounds. Hopkins has shown that one such sulphydrile compound, reduced glutathione, plays an important part in the hydrolytic oxidation-reduction process of the living cell. Voegtlin suggests that a combination of the arsenoxides with such groups and consequent suppression of this vital function may explain the toxic and curative actions of the arsenical derivatives, and that a formation by trypanosomes of the sulphydrile compound in excess of its vital need may be the basis of acquired resistance of trypanosomes. The same probably takes place in the cases of *Leishmania*. Investigations in these directions may lead to discovery of methods of preventing the development of antimony-resistant *Leishmania*.

We have discussed the chemotherapy of antimony from a certain standpoint based chiefly upon the ideas of Ehrlich, Voegtlin and others. Other factors that have to be considered in this connection are the molecular weight of the compounds, their solubility, their dissociation in solution, their surface tension, the hydrogen-ion concentration of the tissue at which they act and various other points which, I am afraid, the time at my disposal will not permit me to discuss.

I shall end here by quoting the remarks of Shortt and Sen which they made in 1925 about urea stibamine: " We

consider the value of urea stibamine has been established as the most efficient drug at present in use in the treatment of kala-azar." This statement remains equally true to-day. To this may be added the remarks of Dodds Price: "I am of opinion that urea stibamine is a most valuable remedy in the resistant types of the disease and I strongly urge that it should be resorted to, if, after a few injections of sodium antimonyl tartrate, a patient does not show marked improvement." I would only add that metallic antimony in a state of fine subdivision should be resorted to in those few cases which may be resistant to urea stibamine and which perhaps do not go beyond 3 per thousand or less.

It will be seen from what I stated that Frankel in his '*Agrentimettel*' is not justified in saying that changes in the molecular structure of antimony compounds do not bring about an increase in their therapeutic properties.

*[Proceedings of a meeting held at the Calcutta Medical Club on the 14th July, 1916]*

Dr. Upendranath Brahmachari read the following paper on "Colloids and other Drugs in the Treatment of Kala-azar," Sir Kailash Chandra Bose being in the chair.

(ABSTRACT)

The remarkable bactericidal properties of electrical colloidal solutions of metallic silver led me to the use of electrargol in the treatment of kala-azar. So far, however, I have not been able to come to any definite conclusion about the therapeutic value of the drug in kala-azar. Electro-mercural and electro-selenium have so far given only negative results.

All of us are aware of the remarkable effects of metallic antimony against the Leishmania and the Trypanosomes. A colloidal preparation of this metal would therefore be an ideal drug in the treatment of kala-azar. Such a preparation would be comparatively free from toxic effects to the human organism and at the same time possess high bacterio-tropic properties against the Leishmania. The following cases fully justify these hopes.

*Case No. I*

Patient A, girl æt. 16, was admitted for treatment of kala-azar. Her spleen extended 6″ below the costal arch and the splenic blood contained an unusually large number of L. D. bodies. Her body weight was 4 stone. She was given 30 injections of colloidal antimony in doses of ·002 grm. to ·003 grm. on successive days. The result of the treatment was as follows :—

(1) Increase of body weight—1st. 10 lb.

(2) Temperature normal for nearly three months.

(3) Spleen gone down by nearly 3 inches.

(4) Disappearance of L. D. bodies from the splenic blood after 20 injections.

(5) R.B.C.—4,100,000, W.B.C.—3,200, Hb.—40% on 29.2.16 (before treatment).

R.B.C.—4,300,000, W.B.C.—3,800, Hb.—44% on 24.3.16 (at commencement of treatment).

R.B.C.—4,300,000, W.B.C.—7,400, Hb.—48% on 14.4.16.

R.B.C.—4,600,000, W.B.C.—7,000, Hb.—58% on 9.6.16 (after treatment).

*Case No. II*

Patient B was admitted into my ward for treatment of kala-azar on 16.2.16. He was cachectic and much emaciated at the time of admission. The spleen extended 3″ below the costal arch and there was a large number of L. D. bodies in the splenic blood. He was at first treated with intramuscular and subsequently with intravenous injections of colloidal metallic antimony. Altogether 20 intravenous injections of the colloid (·002 grm. each) were given. The result of the treatment was as follows :—

(1) Temperature normal.

(2) Increase of body weight—6 lb.

(3) Disappearance of L. D. bodies from the splenic blood.

(4) R.B.C.—2,600,000, W.B.C.—4,800, Hb.—40% on 17.2.16 (before treatment).

R.B.C.—4,300,000, W.B.C.—9,200, Hb.—70% on 30.3.16 (after treatment).

*Case No. III*

Patient T was admitted for treatment of kala-azar. The splenic blood showed presence of L. D. bodies. She had altogether 20 injections of the colloid (·002 grm. each). The result of treatment was as follows :—

(1) Temperature—normal.

(2) Spleen—diminished in size by two inches.

(3) R.B.C—1,600,000, W.B.C.—1,400, Hb.—32% on 1.5.16 (before treatment).

R.B.C.—3,300,000, W.B.C.—5,800, Hb.—54% on 27.6.16 (after treatment).

It will thus be seen that colloidal metallic antimony produced remarkably beneficial effects on the patients.

Another remarkable property possessed by the colloid is that it enables patients, who cannot bear treatment with tartar emetic or sodium antimonyl tartrate due to severe rigor, hyperpyrexia or severe vomiting setting in after the injections, bear treatment with these drugs if at first treated with three or four injections of the metallic colloid. Probably the mild and non-toxic metallic colloid accustoms the patient to bear the subsequent administration of the soluble salts of antimony.

### *Treatment of Kala-azar with Arsenic and Antimony Combined*

In some cases of kala-azar, it has been found that after treatment with antimony, although, generally speaking, there was an all-round improvement, still the hæmoglobin value of the corpuscles could not be raised to the normal. In such cases the combination of soamin with soluble salts of antimony was followed by very good results. In one case the hæmoglobin value was raised from 48 to 64 per cent after 8 injections of soamin, although it was at first almost impossible to raise the hæmoglobin value above 48 per cent with soluble salts of antimony alone. The effect of atoxyl on the treatment of kala-azar was described in a paper of mine published in the *British Medical Journal* some years ago. The paper was entitled *Sporadic Kala-azar in Calcutta with notes of a case treated with Atoxyl*.

It may be stated here that colloidal metallic antimony has been prepared in a stable condition for the first time and been used for the first time by me in kala-azar with the result I have just now mentioned.

All of us are aware that the ointment of metallic antimony introduced by Sir Leonard Rogers produces markedly beneficial results in this disease. I have been able to prepare a non-irritating ointment of tartar emetic and sodium emetic which also yielded similar results in this disease. In one case the ointment was rubbed for nearly a fortnight over the spleen, the liver and the axilla on successive days, as a result of which the spleen was diminished by 2″ in size and the leucocytes rose from 2,600 to 5,600. The patient left hospital before the treatment was completed.

## *Conclusions*

(1) Colloidal metallic antimony has been obtained in a stable suspension for the first time. It produces remarkably beneficial effects in kala-azar.

(2) It is perhaps the least toxic preparation of antimony and its use in kala-azar is followed by tolerance towards the soluble salts of antimony, sodium or potassium antimonyl tartrate, in highly susceptible individuals.

(3) The combination of soamin with antimony rapidly improves the hæmoglobin value of blood in those cases of kala-azar in which it is persistently low.

It is not intended to refer in this paper to my experiences in the treatment of kala-azar with metallic antimony, sodium antimonyl tartrate and tartar emetic. These have been fully stated in my previous papers (*Calcutta Medical Journal*, Oct. and Nov., 1915 and the *Indian Medical Gazette*, Dec., 1915 and January and May, 1916). I would, however, end by quoting what I have stated elsewhere, namely, that " if future observations confirm the view that three or four

injections of metallic antimony are sufficient to bring about a complete and permanent cure of the disease, then we are in possession of a drug as powerful as quinine is for malaria, emetine for amœbic dysentery or salvarsan for syphilis. Its combination with formaldehyde will, perhaps, still more cut short the duration of the desease by the destruction of any antimony-fast parasites that may come into existence." To this I would add that the administration of soamin along with sodium or potassium emetic sometimes leads to a quicker improvement in the blood condition of the patient than is produced by the latter alone.

*N.B.*—The method of preparation of a stable solution of colloidal metallic antimony will be described elsewhere.

## Discussion

Dr. Rai Harinath Ghosh Bahadur remarked that he understood Dr. Brahmachari to speak of the cure of kala-azar by only four or five injections of antimony. But in one of his cases he seemed to have mentioned that at least 20 injections were given to free the blood of the L. D. bodies. He did not thus realise fully the situation. In cases with hepatic enlargement, combination of emetine in one of his cases did accelerate the cure. He was very glad to find that antimony was bringing hopes about the discovery of the curative agent of the fell disease kala-azar.

Dr. Rai Chunilal Bose Bahadur enquired how the colloidal antimony was prepared for Dr. Brahmachari's use. He had read in the *Extra Pharmacopœia* that of all colloidal preparations that of antimony was the most difficult to prepare. Colloidal antimony had already been used in sleeping sickness. But here also the difficulty of obtaining it limited its use.

Dr. Rai Haridhan Dutt Bahadur remarked that the discourse given by Dr. Brahmachari that evening was very

convincing, encouraging, instructive and interesting. The cases which Dr. Brahmachari had cited were certainly very convincing. But he wanted to know whether these successful cases were the only cases treated with colloidal antimony, or these were among a group of several cases similarly treated but not with good results. In private practice he had to deal with a few cases of kala-azar. Soamin and salvarsan treatment both showed marvellous effects in some of his cases, but the effects did not last long and all died after some time. So in the case of antimony treatment it was yet to be seen if the cures were permanent. Neo-salvarsan was also used in three cases and he was struck with the marvellous improvement but these too died after some time. He hoped that Dr. Brahmachari would be able to justify the remarks he made that evening after some time had elapsed. He hoped that as quinine was in malaria so Dr. Brahmachari's colloidal atimony would turn out to be in kala-azar.

Dr. U. N. Brahmachari in reply remarked that he was sorry to find that Dr. Harinath Ghosh did not see the difference between colloidal antimony and metallic antimony in a state of fine subdivision. Three injections of metallic antimony could produce effects which twenty injections of salts of antimony could not do. Regarding Dr. H. D. Dutt's remarks he said that those were the only cases he had treated with colloidal antimony and in all of them he obtained good results. The future of colloidal antimony was great. It was non-irritating. While on the one hand it was the least organotropic, on the other hand it was the most bacteriotropic. But still better preparations might be found hereafter. He previously read a paper about the treatment of kala-azar by atoxyl. With it the red and white corpuscles both increased, but on the last day of the treatment when the patient was about to leave the hospital L. D. bodies were found in his blood. Salvarsan and allied drugs have given

some benefit but this did not last long. He had kept an open mind as his revered professor the late Dr. Bomford used to advise.

Sir Kailash Chandra Bose in thanking the lecturer said that they all heard about the successful treatment of kala-azar by collodial antimony. It had a bright future. It would indeed be a glorious day for India, if an Indian could make further advances in the treatment of kala-azar.

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# PREPARATION OF UREA ANTIMONYL TARTRATE, A NEW COMPOUND

When excess of solid urea is added to a concentrated aqueous solution of hyper-acid-antimonyl tartrate and the mixture concentrated by heating on the water bath and then alcohol added to the mixture, crops of prismatic crystals are obtained. These crystals are soluble in water and only very sparingly soluble in alcohol. They are best purified by being repeatedly washed with absolute alcohol. A solution of the salt gives a faintly acid reaction to litmus paper. On analysis, the proportions of C, H, N and Sb present in the salt, with the water of crystallization, are as follows :—

$$\begin{cases} C = 15{\cdot}20\%, \quad H = 3{\cdot}27\%, \quad N = 4{\cdot}65\%, \quad Sb = 33{\cdot}80\%; \\ \text{Water of crystallization} = 12{\cdot}25\% \end{cases}$$

Calculated from $CO(NH_2)_2\,(C_4H_5.SbO{\cdot}O_6)_2{\cdot}5H_2O$ which is assumed to be the chemical formula of the compound obtained :

$$\begin{cases} C = 15{\cdot}00\%, \quad H = 3{\cdot}33\%, \quad N = 3{\cdot}88\%, \quad Sb = 33{\cdot}33\%; \\ \text{Water of crystallization} = 12{\cdot}50\%. \end{cases}$$

So far as I am aware, there is no reference to this compound in the literature on compounds of urea and antimony.

This salt is being used by me in the treatment of kala-azar. Its toxicity to lower animals seems to be rather low and experiments are in progress to determine its toxic and curative doses.

The solubility curve of the compound in water is shown in the accompanying chart.

I am indebted to Mr. Parimal Sen, M.Sc., for helping me in the preparation of this compound and in working out its solubility curve.

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# A CONTRIBUTION TO THE CHEMISTRY OF CERTAIN NEW AROMATIC ANTIMONIALS

The study of organic antimonials has not been so exhaustive as that of organic arsenicals. In recent years some new organic pentavalent antimonials have been prepared and notable among these is urea stibamine discovered by Brahmachari, which has been found to be of great therapeutic value in the treatment of kala-azar. The reason why much less work has been done with organic antimonials than with arsenicals can be traced mainly to two important causes. First of all, organic antimony compounds are very difficult to prepare and are with few exceptions not crystalline. Secondly, most of them are unstable. This instability limits the formation of various complex antimonials, which has been possible in the case of arsenic. This is especially the case with stibino-benzene compounds as compared with arseno-benzene compounds. Generally speaking, in the case of arsenic, antimony, and bismuth this instability increases as the metallic character of the element becomes more and more pronounced. Thus C-Bi link is less stable than C-Sb link and C-Sb link is less stable than C-As link.

The great difficulty involved in the preparation of aryl antimonials is really a barrier against extensive investigations on this type of compound. This difficulty becomes still greater, as minute impurities and slight variations of physical influences affect the stability of the compounds to a considerable extent, thereby bringing about marked changes in their toxicity and therapeutic properties.

In the *Indian Journal of Medical Research*, the *Indian Journal of Medicine* and the *Calcutta Medical Journal* a series of new organic antimonials were described some time ago by Brahmachari and some of these compounds have been shown to be of great therapeutic value. Another series of new aromatic antimonials have since been investigated in the Brahmachari Research Institute and the following form the first series of such compounds :

1. Disodium *p*-amino-phenyl-stibinate-N-methylene sulphonate.
2. Urea- *p*-amino-phenyl stibinate-N-methylene sulphonateof sodium.
3. Disodium *p*-stibinilate-N-methylene-sulphinate.
4. Urea *p*-amino-phenyl stibinate-N-methylene sulphinate of sodium.
5. *p*-Acetyl-amino-phenyl-stibinate of urea.
6. 1-Acetamino-2-azobenzene-4 : 4-distibinate of sodium.
7. *p*-Hydroxy-phenyl-stibinate of urea.

Some of these compounds, as will be seen from their percentage composition given below, exhibit strong polymerisation whereby three molecules associate together giving rise to more complex molecules.

## Experimental

(1) Disodium *p*-amino-phenyl stibinate-N-methylene sulphonate.

$3(4.SO_3Na.CH_2\text{-}NH.C_6H_4\text{-}SbO_2)H.ONa.$

The starting material in the preparation of this compound is stibanilic acid, which has been prepared by Bart's reaction. Stibanilic acid is neutralised with solution of sodium hydroxide and the sodium salt precipitated by absolute alcohol. The precipitate is then thoroughly washed with absolute alcohol till filtrate is free from alkali. It is next dried in a vacuum dessicator.

Sodium stibanilate is dissolved in water and then formaldehyde solution and $NaHSO_3$ dissolved in water are added to it successively in a flask. The mixture is next heated on a water bath and filtered. The filtrate is treated with excess of alcohol when a bulky precipitate is produced which is washed with alcohol and dried in a porous plate in a vacuum dessicator.

The product is a light coloured powder—easily soluble in water to a perfectly clear solution which gives a faintly acid reaction.

Composition :—

Dried material corresponds to the formula :

$(SO_3Na.CH_2.NH.C_6H_4.SbO)_3O_2(OH)(ONa)$
$= C_{21}H_{22}O_{16}N_3S_3Sb_3Na_4.$

Calculated for $C_{21}H_{22}O_{16}N_3S_3Sb_3Na_4$—
Sb=32·2%, S=8·7%, N=3·8%.

Found—
Sb=32·5%, S=8·5%, N=4·0%.

(2) Urea *p*-amino-phenyl-stibinate-N-methylene sulphonate of sodium.

$3(4\text{-}SO_3Na.CH_2NH.C_6H_4SbO_2)H.OH_3N.CO.NH_2.$

The starting material in this preparation is urea stibamine. Urea stibamine is dissolved in a small quantity of water to which formaldehyde solution and $NaHSO_3$ dissolved in little quantity of water are added in succession. A bulky precipitate is formed on adding the constituents. The mixture is warmed on water bath. It is next filtered and the filtrate precipitated by alcohol. The precipitate is washed with absolute alcohol and then dried over a porous plate in a vacuum dessicator.

The product is a light coloured powder, easily soluble in water and gives a neutral reaction to litmus paper.

Composition :—

Dried material corresponds to the formula :

$SO_3Na.CH_2.NH.C_6H_4SbO)_3O_2(OH)(ONH_3.CO.NH_2)$
$=C_{22}H_{27}O_{17}N_5S_3Sb_3Na_3$.

Calculated for $C_{22}H_{27}O_{17}N_5S_3Sb_3Na_3$ :

Sb=31·1% , S=8·29%, N=6·0%.

Found :

Sb=31·7%, S=8·1%, N=6·3%.

(3) Disodium-*p*-stibanilate-N-methylene sulphinate.
$3(4\text{-}SO_2Na.CH_2.NH.C_6H_4SbO_2)H.ONa$.

Stibanilic acid is treated with NaOH solution and the sodium salt next precipitated by adding absolute alcohol. The precipitate is washed with alcohol to remove the free alkali. The dried sodium salt is then dissolved in a little water and the solution thus obtained treated with sodium formaldehyde sulphoxylate dissolved in little water. A bulky precipitate appears and the whole mixture is warmed on a water-bath when a clear solution is obtained with a small quantity of insoluble impurity. The solution after filtration is slightly concentrated and then precipitated by absolute alcohol. The precipitate is next filtered and dried over a porous plate in a vacuum dessicator.

The product is a light coloured powder readily soluble in water to a perfectly clear solution which is neutral to litmus.

Composition :—

Dried material corresponds to the formula :

$(SO_2Na.CH_2.NH.C_6H_4.SbO)_3O_2(OH)(ONa)$
$=C_{21}H_{22}O_{13}N_3S_3Sb_3Na_4$.

Calculated for $C_{21}H_{22}O_{13}N_3S_3Sb_3Na_4$ :

Sb=33·7%, S=9·0%, N=4%.

Found :

Sb=33·5%, S=9·4%, N=4·2% .

(4) Urea *p*-amino-phenyl stibinate-N-methylene sulphinate of sodium.

$$3(4\text{-}SO_2Na.CH_2.NH.C_6H_4.SbO_2)H.ONH_3.CO.NH_2.$$

Urea stibamine is dissolved in water to which a solution of sodium formaldehyde sulphoxylate is added. A bulky precipitate appears and the whole mixture is well shaken. The mixture is next warmed on a water-bath. A clear solution with a slight sediment at the bottom is obtained which is next filtered. The clear filtrate after concentration is precipitated in cold by absolute alcohol. The precipitate is washed with alcohol, and dried over a porous plate in a vacuum dessicator.

The product is a light coloured powder, readily soluble in water to a perfectly clear reddish solution which is faintly acid to litmus.

Composition :—

Dried material corresponds to the formula :

$$(SO_2Na.CH_2.NH.C_6H_4.SbO)_3O_2(OH)(ONH_3.CO.NH_2)$$
$$= C_{22}H_{27}O_{11}N_6S_3Sb_3Na_3.$$

Calculated for $C_{22}H_{27}O_{14}N_5S_3Sb_3Na_3$ :

$Sb = 32{\cdot}4\%$, $S = 3.65\%$, $N = 6{\cdot}3\%$.

Found :

$Sb = 32{\cdot}0\%$, $S = 3{\cdot}4\%$, $N = 6{\cdot}0\%$.

(5) *p*-Acetyl-amino-phenyl-stibinate of urea.

$$3(4\text{-}CH_3CO.NH.C_6H_3.SbO_2)H.ONH_3.CO.NH_2.$$

The starting material in this preparation is *p*-acetyl-amino-phenyl stibinic acid which is obtained from the corresponding acetyl-phenylene-diamine. The acid is thoroughly washed and the pasty mass is obtained in a semi-dry state by pressing over porous plate. The moist acid is treated with a little urea and then well mixed. The mixture is heated in boiling water when a reddish solution is obtained. A little more water may be added, if necessary, to obtain a clear solution and then warmed. The solution is next

filtered through fluted filter paper and the filtrate precipitated by absolute alcohol. The precipitate is well washed with the same and dried over a porous plate in a vacuum dessicator.

The product is a yellowish powder and dissolves in water to a clear solution which is faintly acid.

Composition :—

Dried material corresponds to the formula :
$(CH_3.CO.NH.C_6H_4.SbO)_3O_2.(OH)(O.NH_3.CO.NH_2)$
$= C_{25}H_{30}O_{11}N_5Sb_3$.

Calculated for $C_{25}H_{30}O_{11}N_5Sb_3$ :
$N = 7{\cdot}48\%$, $Sb = 38{\cdot}4\%$.

Found :
$N = 7{\cdot}9\%$, $Sb = 38{\cdot}0\%$.

(6) 1-Acetamino-2-azobenzene-(4 : 4')-distibinate of sodium.
$CH_3.CO.NH.C_6H_4.N : N.C_6H_3(SbO_3HNa)_2$
$= C_{14}H_{13}O_7N_3Sb_2Na_2$.

The starting materials in the preparation of this compound are acetyl stibanilic acid and stibanilic acid. The former is obtained from acetyl-*p*-phenylene-diamine and the latter by its hydrolysis with alkali. The stibanilic acid is partially dried on a porous plate and suspended in a small quantity of water. The mixture is cooled and treated with excess of $H_2SO_4$ when a clear solution is obtained. Acetyl stibanilic acid dried similarly is weighed and then dissolved in excess of alkali. The former acid solution is then gradually treated with $NaNO_2$ solution till it gives a blue coloration with the starch-iodide paper. The alkaline solution of the acetyl stibanilic acid is also cooled in ice and then gradually added to the diazotised solution. It is then filtered after allowing the little quantity of froth to escape. The sodium salt is then precipitated from the concentrated solution by absolute alcohol—dried over a porous plate in a vacuum dessicator.

The product is a brown powder which dissolves in water giving a clear red solution with neutral reaction.

Composition :—

Calculated for $C_{14}H_{13}O_7N_8Sb_2Na_2$ : N = 6·76%, Sb = 38·6%.
Found : N = 7·0%, Sb = 38·1%.

(7) *p*-Hydroxy-phenyl-stibinate of urea.
$4\text{-}OH.C_6H_4SbO_3H.NH_3.CO.NH_2$.

*p*-Stibanilic acid which is obtained from acetyl-*p*-phenylene-diamine is made into a thick paste with water and an excess of $H_2SO_4$ added, the mixture being cooled. A solution is produced in this way which is well stirred while $NaNO_2$ solution is gradually added till it imparts a blue colour to starch-iodide paper immediately. The mass is next dissolved in alkali after gentle warming to liberate all nitrogen and filtered. The filtrate is reprecipitated with acetic acid. The mixture is filtered and well washed with water. The hydroxy-phenyl-stibinic acid thus obtained, which can also be directly obtained from *p*-amino-phenol by applying Bart's reaction, is then well mixed with little excess of urea and heated on water bath when a clear red solution is obtained. It is then filtered and precipitated by acetone and dried in vacuo over a porous plate.

The product is a yellow powder readily dissolving in water to a perfectly clear solution which is faintly acid to litmus.

Composition :—
Calculated for $C_7H_{11}O_5N_2Sb$ : N = 8·7%, Sb = 37%.
Found : N = 9%, Sb = 37·6%.

The therapeutic value, if any, of these compounds will be reported later on.

*References*

(1) *Indian Journal of Medical Research*, Vol. X, No 2, Oct., 1922; Vol. XI, No. 1, July, 1923; Vol. XI, No. 2, Oct., 1923; Vol. XI, No. 4, April 1924; Vol. XII, No. 1, July 1924; Vol. XII, No. 2, Oct., 1924; Vol. XII, No. 4, April, 1925; Vol. XIII, No. 1, July, 1925; Vol. XIII, No. 3, January, 1926.

(2) *Indian Journal of Medicine*, June, 1926, Sep., 1926.

(3) *Calcutta Medical Journal*, June, 1926, Aug., 1926.

[*Reprinted from the Journal and Proceedings of the Asiatic Society of Bengal (New Series), Vol. XXVI, No. 1, 1930*]

# SYNTHESIS OF A FEW ANTIMONIALS OF THERAPEUTIC INTEREST

This paper contains an account of some organo-metallic antimonials which have been synthesised with the same object in view, as in the case of the compounds described in a previous paper contributed to this *Journal* (Vol. XXV, 1929, No. 1). They are amorphous and extremely difficult to purify. The chemical operations involved in their preparation are given below. As regards their toxicity, we have noticed that in these as in the previous compounds the general rule holds, *viz.*, introduction of sulphoxyl groups lowers the toxicity to a considerable extent with a decrease in the therapeutic value. The nature of the basic portion also affects, to some extent, the stability and the toxicity of the compounds, *viz.*, urea or diethylamine salt is sometimes more stable and less toxic than the corresponding sodium salt. Our object in the preparation of the following compounds is to study these latter effects as well. The compounds are not very stable, though their solutions do not decompose on standing in air for 24 hours. The following is a list of the compounds investigated by us in this paper :—

1. Sodium salt of phenyl-glycine-amide-4-stibinic acid.
2. Urea salt of the same.
3. Diethyl-amine salt of the same.
4. Carbamino-*p*-stibanilate of sodium.
5. Carbamino-*p*-stibanilate of urea.
6. Carbamino-*p*-stibanilate of diethyl amine.

It will be seen that all the above compounds undergo polymerization (see below).

## EXPERIMENTAL

(1) Sodium phenyl-glycine-amide-4-stibinate.

$$
\begin{array}{rl}
 & \mathrm{ONa} \\
 & \vert \\
\mathrm{NH_2.CO.CH_2.NH.C_6H_4.} & \mathrm{SbO} \\
 & \vert \\
 & \mathrm{O} \\
 & \vert \\
\mathrm{NH_2.CO.CH_2.NH.C_6H_4.} & \mathrm{SbO} \\
 & \vert \\
 & \mathrm{O} \\
 & \vert \\
\mathrm{NH_2.CO.CH_2.NH.C_6H_4.} & \mathrm{SbO} \\
 & \vert \\
 & \mathrm{OH}
\end{array}
$$

*p*-Stibanilic acid is dissolved in the requisite quantity of NaOH solution and the concentrated solution of sodium *p*-stibanilate is added gradually to an excess of absolute alcohol, when a precipitate of sodium stibanilate is produced, which is next filtered and washed with absolute alcohol and then dried. 5 grms. of sodium stibanilate are then dissolved in methyl alcohol and treated with chloracetic ester and the whole refluxed for several hours. After the reaction is complete, the methyl alcohol is distilled off and the rest acidified with dilute HCl. The precipitate thus obtained is filtered and washed with water and then treated with concentrated ammonia. After some time, the solution is filtered and the filtrate is reprecipitated by acetic acid, when the glycine amide derivative is obtained which is next washed with distilled water. The precipitate is then dissolved in dilute NaOH, filtered and the filtrate precipitated by adding absolute alcohol. The precipitate is then repeatedly washed with absolute alcohol and dried in a vacuum desiccator.

It is an almost white powder, very easily soluble in water to a perfectly clear solution, which gives neutral reaction with litmus. On warming with dilute alkali it gives out ammonia. The compound prepared according to the above process has been called $X_{10}$, a paper on the thera-

peutics of which has been published in the *Transactions of the Royal Society of Tropical Medicine and Hygiene*. The method of preparation of the compound described here is better than the one originally described by Brahmachari in the *Indian Journal of Medical Research*, 1922.

Composition :—

Dried material corresponds to the formula :

$(NH_2CO.CH_2.NH.C_6H_4SbO)_3.O_2(OH)(ONa)$
$= C_{24}H_{28}O_{10}N_6Sb_3Na$.

Found : Sb = 38·40%, N = 8·72%.
Calculated for $C_{24}H_{28}O_{10}N_6Sb_3Na$ : Sb = 38·17%, N = 8·90%.

This compound is the polymerized antimony analogue of tryparsamide.

(2) Phenyl-glycine-amide-4-stibinate of urea
$(NH_2CO.CH_2.NH.C_6H_4.SbO)_3O_2(OH)(ONH_3.CO.NH_2)$.

Phenyl-glycine-amide-4-stibinic acid, as obtained in the previous experiment, is made into a paste with little water, and then well mixed with an excess of urea. The whole is then warmed for some time when the acid gradually dissolves to a reddish solution, yielding a urea salt. The solution is then filtered through a Buchner funnel, and the clear filtrate is precipitated by acetone. The precipitate thus obtained is dried in a vacuum desiccator after washing well with absolute alcohol.

The product is a light coloured powder easily dissolving in water to a perfectly clear solution which is neutral to litmus.

Composition :—

Dried material corresponds to the formula :

$(NH_2CO.CH_2.NH.C_6H_4.SbO)_3O_2(OH)(ONH_3.CO.NH_2)$
$= C_{25}H_{33}O_{11}N_8Sb_3$.

Found : Sb = 36·58%. N = 11·55%.
Calculated for $C_{25}H_{33}O_{11}N_8Sb_3$ : Sb = 36·69%, N = 11·41%.

(3) Phenyl-glycine-amide-4-stibinate of diethyl amine

$$(NH_2CO.CH_2.NH.C_6H_4.SbO)_3O_2(OH).OH_2N(C_2H_5)_2.$$

The starting material in the preparation of this compound is the same as in the previous cases. This is well mixed with a small quantity of water, and to the mixture a 30 per cent solution of diethylamine in water is gradually added, shaking it very well at the same time. Almost a clear concentrated solution is thus obtained, which is filtered and the reddish filtrate is poured drop by drop into 5 times its volume of absolute alcohol. A voluminous precipitate is produced, which is allowed to settle down for scme time and then filtered. The precipitate is washed well with absolute alcohol and then dried in a vacuum desiccator.

It is a light grey powder easily dissolving in water to a clear solution which is neutral to litmus.

Composition :—

Dried material corresponds to the formula :

$$(NH_2CO.CH_2.NH.C_6H_4.SbO)_3O_2(OH).OH_2N(C_2H_5)_2$$
$$= C_{28}H_{40}O_{10}N_7.Sb_3.$$

Found : Sb = 36·42%, N = 9·71%.
Calculated for $C_{28}H_{40}O_{10}N_7Sb_3$ : Sb = 36·21%, N = 9·85%.

(4) Sodium carbamino-*p*-stibanilate

$$\begin{array}{r} ONa \\ | \\ NH_2.CO.NH.C_6H_4.SbO \\ | \\ O \\ | \\ NH_2.CO.NH.C_6H_4.SbO \\ | \\ O \\ | \\ NH_2.CO.NH.C_6H_4.SbO \\ | \\ OH \end{array}$$

The starting material in the preparation of this compound is sodium *p*-stibanilate, produced by neutralising *p*-stibanilic acid with NaOH solution, the acid itself being obtained by

hydrolysing acetyl-*p*-stibanilic acid and which is a product of Bart's reaction applied to acetyl-*p*-phenylene-diamine. Five grms. of sodium stibanilate thus obtained are dissolved, at low temperature, in glacial acetic acid. To this well-cooled mixture are gradually added about 4 grms. of potassium cyanate and the mixture well stirred till a clear solution is obtained. The solution is then allowed to remain in this state for several hours. The mixture is then diluted with water and well stirred. Concentrated HCl is then gradually added which dissolves the unreacted *p*-stibanilic acid and precipitates the carbamino derivative as a voluminous mass, which is then filtered and washed with water. The wet precipitate is then dissolved in the requisite quantity of dilute NaOH solution and the reddish solution thus obtained is filtered. The filtrate is precipitated by absolute alcohol and the precipitate washed with the same and then dried in a vacuum desiccator.

The product is almost a white powder readily dissolving in water to a clear solution which is neutral to litmus.

Composition :—

Dried material corresponds to the formula :

$(NH_2.CO.NH.C_6H_4.SbO)_3O_2.OH.ONa.$

$= C_{21}H_{22}O_{10}N_6Sb_3Na.$

Found : Sb=39·62%, N=9·29%.

Calculated for $C_{21}H_{22}O_{10}N_6Sb_3Na$ : Sb=39·95%, N=9·32%.

(5) Carbamino-*p*-stibanilate of urea

$(NH_2.CO.NH.C_6H_4.SbO)_3.O_2.OH.ONH_3.CO.NH_2.$

Carbamino-*p*-stibanilic acid, as obtained in the previous case, is made into a paste with a little water and then well mixed with a slight excess of urea. The mixture is then warmed on a water bath when the acid gradually dissolves to a clear solution. The solution is next filtered and the filtrate precipitated by acetone.

It is a light grey powder which dissolves easily in water giving a neutral solution..

Composition :—

Dried material corresponds to the formula :

$$(NH_2.CO.NH.C_6H_4.SbO)_3.O_2.OH.ONH_3.CO.NH_2$$
$$= C_{22}H_{27}O_{11}N_8Sb_3.$$

Found : Sb=38·50%, N=11·85%.

Calculated for $C_{22}H_{27}O_{11}N_8Sb_3$ : Sb=38·34%, N=11·92%.

(6) Carbamino-*p*-stibanilate of diethyl amine

$$(NH_2.CO.NH.C_6H_4.SbO)_3.O_2.OH.ONH_2.(C_2H_5)_2.$$

As in the previous experiment a paste is made by mixing carbamino-*p*-stibanilic acid with little water to which is then gradually added a 35 per cent solution of diethyl-amine in water. The precipitate gradually dissolves, giving a clear solution which is filtered, and the filtrate reprecipitated by acetone.

It is a pale greyish powder which dissolves readily in water.

Composition :—

Dried material corresponds to the formula :

$$(NH_2.CO.NH.C_6H_4.SbO)_3.O_2.OH.ONH_2.(C_2H_5)_2$$
$$= C_{25}H_{34}O_{10}N_7Sb_3.$$

Found : Sb=37·62%, N=10·31%.

Calculated for $C_{25}H_{34}O_{10}N_7Sb_3$ : Sb=37·81%, N=10·29%.

*References*

1. Journal and Proceedings of the Asiatic Society of Bengal (New Series), Vol XXV, No. 1, 1929.

2. Transactions of the Royal Society of Tropical Medicine and Hygiene, Vol. XXIII, No. 6, pp. 617-622, April, 1930.

[*Reprinted from the Indian Journal of Medicine, Vol. XII, Feb., 1931*]

# SYNTHESIS OF SODIUM N-PHENYL-GLYCINE-AMIDE-4-STIBINATE (ANTIMONY ANALOGUE OF TRYPARSAMIDE)

This paper gives the method of preparation of one of a series of new antimonials of therapeutic interest which were under publication in the *Journal and Proceedings of the Asiatic Society of Bengal*. (*Vide page 390.*)

The above compound has been found to be of therapeutic value in the treatment of kala-azar, and a series of cases has been published in the *Transactions of the Royal Society of Tropical Medicine and Hygiene*, Vol. XXIII, No. 6, pp. 617-622, April, 1930. A paper containing a further series of cases of kala-azar treated with this compound is under preparation.

[*N.B.*—Another method of preparation of the above compound by Brahmachari was described in the *Indian Journal of Medical Research*, October, 1922.—Ed.]

[*Reprinted from the Journal of Tropical Medicine and Hygiene, August 15, 1931*]

# THE INTENSIVE ANTIMONIAL TREATMENT OF KALA-AZAR

## PART I

In the *Indian Journal of Medical Research* (1925), Brahmachari described a series of cases of kala-azar treated by an intensive course with urea stibamine, which consisted of injections given daily or on alternate days or of multiple injections given on the same day during a course ranging from thirty-six hours to seven days.

Since the publication of the above paper one hundred and twenty-five more cases in which the intensive treatment by daily administration of the above drug produced similarly satisfactory results, consisting of lessening of the period of treatment which extended from seven to ten days and without any untoward symptoms due to the short-interval injections, have been recorded.

The method of treatment by daily injections was subsequently followed by Napier and Mullick with another antimonial, *neo-stibosan,* and they reported that they likewise had got satisfactory results in their cases.

It may at once be mentioned here that cases of kala-azar vary most markedly in their response to specific treatment, as has been pointed out in this Journal and elsewhere. This variability is perhaps more noticeable in kala-azar than in any other protozoal disease. It is very important to remember this, as otherwise medical men may be tempted to believe that a case that has had a week's or ten days' treatment with a pentavalent aromatic anti-

monial is cured, or one may be disappointed if one finds that a particular case is not responding as quickly as one would expect from a study of the reported cases in which cure has been recorded by a short course of treatment.

As illustrations of what may be regarded as *easily cured* cases of the disease, the notes of a few cases that recently came under our observation are appended here :—

(*i*) Patient, æt. 6, was admitted into the wards of the Chittaranjan Hospital with history of fever with double rise of temperature for 9 months. Spleen extended 3½ in. below the costal arch. L. D. bodies were found on spleen puncture. Blood examination showed R. B. C.—1,200,000, W.B.C.—2,872 and Hb.—40 per cent. Patient was given daily intravenous injections of urea stibamine for 7 days in doses of (1) ·075g., (2) ·1g., (3) ·15g., (4) ·2g., (5) ·2g., (6) ·2g., the total amount being ·925 gramme, after which the treatment was stopped. Patient was kept under observation for one month and a half. At the time of discharge, spleen could not be felt below the costal arch, no L. D. bodies could be found on spleen puncture and W. B. C. count was 8,000 per c.mm. Patient was free from fever during the period of observation after the course of treatment.

(*ii*) Patient S was treated in the Health Association, Ward No. 4, Calcutta Municipality, with history of fever with daily double rise of temperature for 9 months. Spleen extended 4½ in. below the costal margin. Patient was given daily intravenous injection of urea stibamine for 6 days in doses of (1) ·025g., (2) ·025g., (3) ·025g., (4) ·025g., (5) ·05g., (6) ·05g., the total amount being ·2 gramme, after which the treatment was stopped. Patient was kept under observation for three months. At the time of discharge, spleen could not be felt below the costal margin. Patient was free from fever during the whole period of observation after the course of treatment.

(*iii*) Patient D, æt. 8, was admitted in the Chittaranjan Hospital with history of fever with daily double rise of temperature for 9 months. Spleen extended 4 in. below the costal arch and liver just palpable. L. D. bodies were found on spleen puncture. Blood examination showed R.B.C.—2,800,000, W.B.C.—3,120 and Hb.—45 per cent. Patient was given daily intravenous injection of urea stibamine for 7 days in doses of (1) ·025g., (2) ·05g., (3) ·1g., (4) ·1g., (5) ·1g., (6) ·1g., (7) ·1g., the total amount being ·575 gramme, after which treatment was stopped. Patient was kept under observation for 33 days. At the time of discharge, spleen could not be felt below the costal arch, no L. D. bodies could be found on spleen puncture and W.B.C. were 6,552 per c.mm. Patient was free from fever during observation.

(*iv*) Patient P, æt. 29, was admitted in the Chittaranjan Hospital with history of fever with daily double rise of temperature for one year and two months. Spleen extended $3\frac{1}{2}$ in. below the costal arch. Blood examination showed R.B.C.—3,100,000, W.B.C.—3,432 and Hb. —45 per cent. L. D. bodies were found on spleen puncture. Patient was given daily intravenous injections of urea stibamine for 8 days in doses of (1) ·05g., (2) ·075., (3) ·1g., (4) ·1g., (5) ·1g., (6) ·15g., (7) ·2g., (8) ·2g., the total amount being ·975 gramme, after which the treatment was stopped. Patient was kept under observation for 27 days. At the time of discharge, spleen could not be felt below the costal arch, no L. D. bodies could be found on spleen puncture and W.B.C. were 6,573 per c.mm. Patient was free from fever during observation.

(*v*) Patient K, æt. 30, was admitted in the Chittaranjan Hospital with history of fever with daily double rise of temperature for 7 months. He had previously 12 injections of neo-stibosan. Spleen extended $4\frac{1}{2}$ in. below the costal margin at the time of admission. Blood examination

showed R.B.C.—3,600,000, W.B.C.—2,184 and Hb.—65 per cent. L. D. bodies were found on spleen puncture. Patient was given daily intravenous injections of urea stibamine for 9 days in doses of (1) ·025g., (2) ·05g., (3) ·05g., (4) ·1g., (5) ·15g., (6) ·2g., (7) ·15g., (8) ·2g., (9) ·2g., the total quantity being 1·125 grammes, after which treatment was stopped. Patient was under observation for two months. At the time of discharge, spleen could not be felt below the costal arch, no L. D. bodies could be found on spleen puncture and W.B.C. was 5,800 per c.mm.

(*vi*) Patient N, æt. 7, was admitted in the Chittaranjan Hospital with history of fever with daily double rise of temperature for 8 months. Both spleen and liver extended 2 in. below the costal arch. L. D. bodies were found on spleen puncture. Blood examination showed R.B.C.—1,720,000, W.B.C.—2,500 and Hb.—35 per cent. Patient was given daily intravenous injections of urea stibamine for 9 days in doses of (1) ·025g., (2) ·05g., (3) ·075g., (4) ·1g., (5) ·1g., (6) ·1g., (7) ·1g., (8) ·1g., (9) ·1g., the total amount being ·75 gramme, after which the treatment was stopped. The patient was kept under observation for two months. At the time of discharge, spleen could not be felt below the costal arch, no L. D. bodies could be found on spleen puncture, and W.B.C. count was 6,056 per c.mm. There was no fever during the period of observation.

On the other hand, there are cases in which a prolonged course of treatment with any antimonial, aromatic or otherwise, only slowly influences the course of the disease. It is to these cases that attention of research workers should be directed at the present day, especially in view of the fact that these cases are frequently not properly recorded by those who advocate a particular antimonial preparation, which failed to act quickly in them.

It has been frequently held that early cases are easily amenable to treatment—a view originally expressed by

Brahmachari in a paper published in the *Indian Journal of Medical Research* in 1924 under the title of "Value of Urea Stibamine in the Treatment of Early Kala-azar." Further experience has, however, shown that while this may be so in a large number of patients, there are certain cases which are refractory from the very beginning.

Further, in an early case, one sometimes observes that a few weeks' delay in commencing specific treatment helps in bringing about a cure more quickly than immediately starting the treatment.

This brings one to the question of production of antibodies in the treatment of kala-azar. Just as in the case of treatment of syphilis with an arsenical, or of malaria with quinine, little work has been done in the production of antibodies in the treatment of kala-azar with an antimonial.

The object of this paper is to indicate that while the intensive treatment of kala-azar with urea stibamine cuts short the course of treatment of the disease as quickly as any other antimonial that has now been put on the market, it would be misleading to assert that such brilliant results are to be met with in *all* cases, whatever that antimonial preparation may be.

## REMARKS

The mechanism of response of leishmania to an antimonial preparation is a very complicated one. While an aromatic pentavalent antimonial, such as urea stibamine, brings about sterilization of an infected individual in a much shorter time than tartar emetic, it must, at the same time, be admitted that, with any such compound, the time required for sterilization is variable in the case of different individuals. What is the mechanism of this variability? This constitutes an important line of research and will be referred to in a subsequent paper.

## *References*

Brahmachari, U. N. (1924). Chemotherapy of Antimonial Compounds in Kala-azar Infection. Part XI.—The Value of Urea Stibamine in the Treatment of Early Kala-azar. *Indian Journal of Medical Research*, Oct., 1924.

Brahmachari, U. N. & Maity, B. B. (1925). Chemotherapy of Antimonial Compounds in Kala-azar Infection. Part XIV.—Observations on a series of cases of Kala azar treated with Urea Stibamine during a course of 32 hours to 7 days. *Ibid.*, April, 1925.

[Reprinted from the Journal of Tropical Medicine and Hygiene, January 2, 1933]

# THE INTENSIVE ANTIMONIAL TREATMENT OF KALA-AZAR

## PART II

This paper is a continuation of a previous paper by Brahmachari and a co-worker published in this Journal (1931). It gives a collective series of 31 cases of kala-azar treated with urea stibamine by the intensive method, in addition to those previously recorded. The cases were treated in the Tropical Diseases Ward of the Carmichael Medical College Hospitals, the Kala-azar Ward of the Chittaranjan Hospital, the Campbell Hospital, Calcutta, and in the Out-Patient Department of the Chittaranjan Hospital, Calcutta. It was intended to test the value of the drug by the intensive method independently and give a collective report. The present paper gives a report of cases treated up to the time of writing. Further observations are still in progress, and will be reported in another series.

(*a*) *Notes on Cases treated in the Tropical Diseases Ward of the Carmichael Medical College Hospitals, and in the Kala-azar Ward of the Chittaranjan Hospital, Calcutta.*

Case No. 1.—J. N. D., æt. 19, was admitted on 22-9-32. History of irregular fever for about nine months. Patient anæmic, liver extended $1\frac{1}{2}$ in. and spleen 3 in. below the costal arch. Patient developed pleurisy in hospital before he was treated for kala-azar. Spleen puncture: L. D. bodies present. After the patient was cured of

pleurisy, he was treated with urea stibamine injected intravenously on successive days from 4-10-32. The following were the doses given: (1) 0·05 g., (2) 0·05 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·15 g., (7) 0·15 g., (8) 0·15 g., (9) 0·15 g. and (10) 0·2 g. Altogether ten injections were given with a total amount of 1·2 grms. The temperature came down to normal after the first injection and remained so up to the day of discharge from hospital. No reactions were observed during treatment. At the time of discharge, spleen could not be felt below the costal margin and no L. D. bodies were found on spleen puncture. Patient remained in hospital for two months after the completion of treatment.

CASE NO. 2.—N., æt. 36, was admitted on 3-9-32. History of irregular fever for about a year with double rise of temperature, pyorrhœa alveolaris and bleeding from gums present. Patient anæmic. Liver extended ½ in. and spleen 6 in. below the costal arch in the mid-clavicular line. Spleen puncture: L. D. bodies present. Patient was treated with urea stibamine injected intravenously on successive days from 7-9-32. The following were the doses: (1) 0·05 g., (2) 0·1g., (3) 0·1 g., (4) 0·1 g., (5) 0·15 g., (6) 0·15 g., (7) 0·15 g., (8) 0·15 g., (9) 0·15 g., (10) 0·2 g. and (11) 0·2 g. Altogether eleven injections were given with a total amount of 1·5 grms. The temperature came down to normal after the second injection and remained so up to the day of discharge from hospital. No reactions were observed during treatment. At the time of discharge, spleen extended 2 in. below the costal margin and no L. D. bodies were found on spleen puncture. The patient remained in hospital for two months after the completion of treatment.

CASE NO. 3.—A. C. P., æt. 20, was admitted on 31-8-32. History of irregular fever for 1 year. About three months ago patient had general anasarca. Liver was tender, extending 1 in. and spleen 6 in. below

the costal arch. Spleen puncture : L. D. bodies present. Patient was treated with urea stibamine injected intravenously on successive days from 7-8-32. The following were the doses : (1) 0·05 g., (2) 0·1 g., (3) 0·1 g., (4) 0·1 g., (5) 0·15 g., (6) 0·15 g., (7) 0·15 g., (8) 0·15 g. and (9) 0·2 g. Altogether nine injections were given with a total amount of 1·15 grms. The temperature came down to normal after the second injection and remained so up to the day of discharge from hospital. At the time of discharge, spleen could just be felt below the costal margin, but no L. D. bodies were found on spleen puncture. Patient's general condition very much improved. Patient remained in hospital for two months after the completion of treatment.

CASE NO. 4.—A. M., æt. 14, was admitted on 22-8-32. History of irregular fever. Patient was ill-nourished. At the time of admission, ascites and œdema of the feet were present with old scars over the abdomen in the splenic area. Liver was hard, its margin extending ½ in. below the costal margin and spleen was very hard, reaching the level of the umbilicus and ½ in. from the middle line to the right. Spleen puncture : L. D. bodies present. There was presence of albumin in the urine. Patient was treated with urea stibamine injected intravenously on successive days from 28-8-32. During treatment ascites and œdema disappeared. The following were the doses : (1) 0·05 g., (2) 0·05 g., (3) 0·05 g., (4) 0·1 g., (5) 0·1 g., (6) 0·1 g., (7) 0·1 g., (8) 0·15 g., (9) 0·15 g., (10) 0·1 g., (11) 0·1 g. and (12) 0·15 g. Altogether twelve injections were given, with a total amount of 1·15 grms. The temperature came down to normal after the fourth injection and remained so up to the day of discharge from hospital. No reactions were observed during treatment. At the time of discharge, spleen extended one inch below the costal margin and no L. D. bodies were found on spleen puncture. Patient remained in hospital for two months after the completion of treatment.

CASE NO. 5.—J. A. M., æt. 25, was admitted on 2-9-32. History of irregular fever and general anasarca. At the time of admission, patient was anæmic with œdema of the face and limbs. Liver was hard and extended 2 in. and spleen, also very hard, extended 7 in. below the costal margin in mid-clavicular line and 4 in. to the right of the middle line. Spleen puncture: L. D. bodies present. Patient was treated with urea stibamine injected intravenously on successive days from 7-9-32. The following were the doses: (1) 0.05 g., (2) 0·1 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·15 g., (7) 0·15 g., (8) 0·15 g., (8) 0·15 g., (9) 0·15 g., (10) 0·15 g. and (11) 0·2 g. Altogether eleven injections with a total amount of 1·35 grms. were given. The temperature came down to normal after the first injection and remained so up to the day of discharge from hospital. No reactions were observed during treatment. At the time of discharge, spleen could be felt about 2 in. below the costal margin, but no L. D. bodies were found on spleen puncture. Patient remained in hospital for one month after the completion of treatment.

CASE NO. 6.—G. N. K., æt. 14, was admitted on 29-8-32. History of irregular fever for about three years. Spleen extended 9½ in. and liver enlarged to the finger's breadth below the costal arch. Spleen puncture: L. D. bodies present. Patient was treated with urea stibamine injected intravenously on successive days from 6-9-32. The following were the doses: (1) 0·05 g., (2) 0·05 g., (3) 0·05 g., (4) 0·05 g., (5) 0·1 g., (6) 0·1 g., (6) 0·1 g., (7) 0·1 g., (8) 0·15 g., (9) 0·15 g. and (10) 0·15 g. Altogether ten injections with a total amount of 0·95 g. were given. The temperature came down to normal after the first injection, and remained so up to the day of discharge from hospital. No reactions were observed during treatment. At the time of discharge, spleen was 2 in.

below the costal margin and no L. D. bodies were found on spleen puncture. Patient remained in hospital for one month after the completion of treatment.

CASE NO. 7.—T. G., æt. 20, was admitted on 30-6-32. History of irregular fever with double rise of temperature during the first seven months. Spleen extended $5\frac{1}{2}$ in. below the costal margin and liver not enlarged. Patient moderately anæmic. Spleen puncture: L. D. bodies present. Patient was treated with urea stibamine, injected intravenously on successive days from 6-9-32. The following were the doses: (1) 0·15 g., (2) 0·15 g., (3) 0·15 g., (4) 0·15 g., (5) 0·15 g., (6) 0·15 g., (7) 0·2 g., (8) 0·2 g., (9) 0·2 g. and (10) 0·2 g. Altogether ten injections were given with a total amount of 1·7 grms. The temperature came down to normal after the second injection and remained so up to the day of discharge from hospital. No reactions were observed during treatment. At the time of discharge, spleen was $1\frac{1}{2}$ in. below the costal margin, but no L. D. bodies were found on spleen puncture. Patient remained in hospital for one month after the completion of treatment.

CASE NO. 8.—B. K. D., æt. 25, was admitted on 26-9-32. History of continued fever for five months and of double rise of temperature for one month. Spleen extended $5\frac{1}{2}$ in. below the costal margin and liver was just palpable. Spleen puncture: L. D. bodies present. Patient was treated with urea stibamine, injected intravenously on successive days from 28-9-32. During this period, he had an attack of influenza and the injections were stopped for the time being. After the subsidence of bronchitis, injections of urea stibamine were continued from 21-10-32 to 24-10-32. The following were the doses: (1) 0·025 g., (2) 0·05 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·15 g., (7) 0·1 g., (8) 0·15 g., (9) 0·2 g. and (10) 0·2 g. Altogether ten injections with a total amount of 1·175 grms. were given. The temperature came down to normal after the third injection and

remained so up to the day of discharge from hospital. No reactions were observed during treatment. At the time of discharge, spleen was 2 in. below the costal margin, and no L. D. bodies were found on spleen puncture. Patient remained in hospital for one month after the completion of treatment.

CASE No. 9.—B. P. R., æt. 15, was admitted on 20-9-32. History of fever of an intermittent type, occurring irregularly for about one year with bleeding from the gums. Spleen extended $5\frac{1}{2}$ in. and liver $1\frac{1}{2}$ in. below the costal margin. Spleen puncture: L. D. bodies present. Patient was treated with urea stibamine injected intravenously on successive days from 22-9-32. The following were the doses given: (1) 0·1 g., (2) 0·1 g., (3) 0·15 g., (4) 0·15 g., (5) 0·2 g., (6) 0·2 g., (7) 0·2 g. and (8) 0·2 g. Altogether eight injections with a total amount of 1·3 grms. were given. The temperature came down to normal during treatment and no reactions were observed thereafter. At the time of discharge, spleen was $1\frac{1}{2}$ in. below the costal margin and no L. D. bodies were found on spleen puncture. Patient remained in hospital for two months after the completion of treatment.

CASE No. 10.—S. N. M., æt. 30, was admitted on 10-10-32. History of remittent type of fever for 15 days. Spleen extended about 2 in. below the costal margin and liver was just palpable. Widal was negative. Spleen puncture: L. D. bodies present. Patient was treated with urea stibamine injected intravenously on successive days from 17-10-32. The following were the doses: (1) 0·05 g., (2) 0·1 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g. and (6) 0·1 g. Altogether six injections were given with a total amount of 0·55 g. The temperature came down to normal after the third injection and remained so up to the day of discharge from hospital. No reactions were observed during treatment. Spleen and liver could not be felt below the costal

margin, and no L. D. bodies were found on spleen puncture. Patient remained in hospital for one month after the completion of treatment.

CASE NO. 11.—M., æt. 16, was admitted on 19-10-32. History of irregular fever for about 8 months and anæmia with œdema of the lower extremities for about one month. Spleen extended $5\frac{1}{2}$ in. below the costal margin. Spleen puncture: L. D. bodies present. Patient was treated with urea stibamine injected intravenously on successive days from 20-10-32. The following were the doses: (1) 0·025 g., (2) 0·025 g., (3) 0·05 g., (4) 0·1 g., (5) 0·1 g., (6) 0·1 g., (7) 0·15 g., (8) 0·15 g., (9) 0·2 g. and (10) 0·2 g. Altogether ten injections were given with a total of 1·1 grms. The temperature came down to normal after the sixth injection and remained so up to the day of discharge from hospital. No reactions were observed during treatment. Spleen could just be felt below the costal margin, but no L. D. bodies were found on spleen puncture and there was neither anæmia nor any œdema of the legs. Patient remained in hospital for one month after the completion of treatment.

### (b) *Notes on Cases treated in the Medical Wards of the Campbell Hospital, Calcutta*

CASE NO. 1.—S. B., H.M., æt. 24. History of irregular intermittent fever for nearly 4 months. At the time of admission, liver extended $\frac{3}{4}$ in. and spleen $2\frac{1}{2}$ in. below the costal arch in the mid-clavicular line. Muscular wasting fairly marked and there was slight pitting on the skin on the tibiæ.

*Blood Report.*—R. B. C.—3.900,000, W.B.C.—4,000, polymorphonuclears—61 per cent, lymphocytes—37 per cent, large mononuclears—2 per cent, eosinophiles—nil. Urea stibamine precipitation test strongly positive.

*Treatment.*—Daily injection of urea stibamine. 0·05 g., 1 g. and 2 g. doses, eleven injections in all, total quantity of urea stibamine given being 1·75 grms.

*Result.*—Fever disappeared after the third injection. Liver and spleen were no longer palpable. Patient was kept under observation for a period of three weeks after the last injection. At the time of discharge, the condition was as follows: Liver and spleen could not be felt below the costal margin. Blood-count: R. B. C. —4,000,000, W.B.C.—6,500, polymorphonuclears—68 per cent, lymphocytes—26 per cent, large mononuclears—5 per cent, eosinophiles—1 per cent. Urea stibamine test absolutely negative. Total gain in weight was nearly one stone.

CASE NO. 2—Dilmaya, H.F., æt. 20.

*Previous History.*—Irregular intermittent fever for nearly 7 months. Patient was cachectic with much muscular wasting, anæmia and some œdema of the legs. Liver extended 3 in. and spleen 5 in. below the costal arch in the mid-clavicular line.

*Blood Report.*—R.B.C.—1,750,000, Hb.—30 per cent, W.B.C.—2,000, polymorphonuclears—38 per cent, lymphocytes—40 per cent, large mononuclears—2 per cent, eosinophiles—nil. Formalin and urea stibamine precipitation tests strongly positive.

*Treatment.*—Bi-weekly injections of urea stibamine from 0·05 to 0·15 g., eight injections in all containing one gram of urea stibamine were given, preceded by intravenous injections of calcium chloride 10 per cent sol. 2 c.c. each. Eight injections of hepatrat. 3 c.c. each were also given. As œdema disappeared and the general condition of the patient improved, she was given daily injections of urea stibamine, thirteen injections in all, containing 2·5 grms. of urea stibamine.

*Result.*—Fever disappeared after the third injection of urea stibamine. Two weeks after the last injection, the

condition was as follows: Liver was just palpable and spleen, one inch below the costal margin, was hard and appeared to be markedly fibrosed. Blood Picture : R.B.C. —3,500,000, W.B.C.—7,000, polymorphonuclears—60 per cent, lymphocytes—36 per cent, large mononuclears—2 per cent, eosinophiles—2 per cent. Formalin and urea stibamine tests were negative. Total gain in weight was 1st. 10lb.

CASE NO. 3.—S. B. Debi, H.F., æt. 30. Previous history of irregular fever 5½ months, ranging from 102·4° to 97·6° F. General condition was poor with much anæmia, dilated heart and general anasarca. Liver 2½ in. and spleen 4½ in. below the costal margin.

*Blood Report.*—R.B.C.—1,125,000, Hb.—20 per cent, W.B.C.—2,000, polymorphonuclears—62 per cent, lymphocytes—32 per cent, large mononuclears—6 per cent. Formalin and urea stibamine tests were strongly positive.

*Treatment.*—The patient was first digitalized and intravenous injections of calcium chloride, 10 per cent sol. 2 c.c. each, were given for 12 days till the œdema nearly disappeared. Then she was put on daily injections of urea stibamine in 0·05 g., 0·1 g., 0·15 g. and 0·2 g. doses. Altogether fifteen injections were given, amounting to a total of 2·35 grms. of urea stibamine. Fever disappeared after the sixth injection. One week after the last injection, when the patient left hospital, the condition was as follows: Liver was just palpable and spleen ½ in. below the costal margin. Blood Picture: R.B.C.—2,500,000, Hb.—45 per cent, W.B.C.—5,000, polymorphonuclears—65 per cent, lymphocytes—30 per cent, large mononuclears—5 per cent. Formalin and urea stibamine tests were negative. Total gain in weight was 5 lb.

CASE NO. 4.—S. N. Das, H.M., æt. 12.

*Previous History.*—Irregular intermittent fever for the last 6 months. At present the temperature is between 100° and 98° F. Liver 2¼ in. and spleen 3 in. below the costal

margin at the mid-clavicular line. Muscular wasting is fairly well-marked and so also the black pigmentation of the skin.

*Blood Report.*—R.B.C.—2,600,000, W.B.C.—2,400, polymorphonuclears—52 per cent, lymphocytes—38 per cent, large mononuclears—8 per cent, eosinophiles—2 per cent. Formalin and urea stibamine tests were strongly positive.

*Treatment.*—Daily injections of urea stibamine [0·05 g. three injections; 0·1 g. two injections; 0·15 g. five injections; 0·2 g. three injections] were given, totalling 1·7 grms. of urea stibamine.

*Result.*—Fifteen days after the last injection blood-count showed: R.B.C.—3,300,000, W.B.C.—8,000, polymorphonuclears—56 per cent, lymphocytes—32 per cent, large mononuclears—2 per cent, eosinophiles—10 per cent. Urea stibamine test was slightly positive and the spleen was 1½ in. below the costal margin. There was no fever. He was given another course of ten injections of 0·2 g. of urea stibamine daily. At the end of it the spleen was just palpable at the costal margin and urea stibamine test was negative. The total gain in weight was about half a stone.

### (c) *Notes on Cases treated in the Out-patient Department of Chittaranjan Hospital, Calcutta*

CASE NO. 1.—S., H.F., æt. 38, came under treatment with history of fever for nearly 6 months and with double rise of temperature for 20 days. Spleen measured 3½ in. below the costal arch. Liver was not palpable. Aldehyde test was strongly positive. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·05 g., (2) 0·05 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·15 g., (7) 0·15 g. and 0·15 g. The number of injections was eight and the total quantity was 0·85 g. The temperature came down to normal after the second injection and remained normal during observation. No reactions were

observed during treatment. Spleen could not be felt below the costal margin and the patient's general condition was satisfactory after the completion of treatment.

CASE No. 2.—M., H.M., æt. 12, came under treatment with history of fever with double rise of temperature for nearly 5 months. Spleen extended 5½ in. below the costal arch and liver was just palpable. Aldehyde test was fairly positive. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·05 g., (2) 0·05 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·15 g., (7) 0·15 g., (8) 0·15 g., (9) 0·15 g. and (10) 0·15 g. The number of injections was ten and the total quantity was 1·25 grms. The temperature came down to normal after the third injection and remained so while he was under observation. No reactions were observed during treatment. Spleen was only ½ in. below the costal margin and liver was normal. The patient's general condition very much improved one month and a half after the completion of treatment.

CASE No. 3.—K., H. M., æt. 17, came under observation with history of continuous fever for about one month and a half. Spleen was 2½ in. below the costal arch and liver not palpable. Aldehyde test was negative. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·05 g., (2) 0·5 g., (3) 0·5 g. and (4) 0·5 g. The number of injections was four and the total quantity was 0·2 g. The temperature came down to normal after the first injection. No reactions were observed during treatment. Spleen could not be felt and patient's general condition was satisfactory.

CASE No. 4.—S., H. F., æt. 12, came under treatment with history of occasional attacks of fever for about one year and with double rise of temperature for 10 days. Spleen measured 3½ in. below the costal arch and liver normal. Aldehyde test was fairly positive. Patient was given daily intravenous injections of urea stibamine in the following

doses: (1) 0·125 g., (2) 0·5 g., (3) 0·05 g., (4) 0·1 g., (5) 0·1 g., (6) 0·1 g., (7) 0·1 g. and (8) 0·1 g. The number of injections was eight and the total quantity was 0·625 g. The temperature came down to normal after the third injection. No reactions were observed during treatment. Spleen could not be felt and patient's general condition was very satisfactory.

CASE NO. 5.—S., H. M., æt. 5, came under treatment with history of fever for about one year and with double rise of temperature for one month. Spleen measured 4½ in. below the costal arch and liver not palpable. Aldehyde test was strongly positive. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·025 g., (2) 0·025 g., (3) 0·025 g., (4) 0·025 g., (5) 0·025 g. and (6) 0·025 g. The number of injections was six and the total quantity was 0·715 g. The temperature came down to normal after the second injection. No reactions were observed during treatment. Spleen could not be felt below the costal arch and the general condition of the patient improved considerably.

CASE NO. 6.—R., M.M., æt. 25, came under treatment with history of fever for about one year and with double rise of temperature for 3 months. Spleen measured 6 in. below the costal margin and liver was not palpable. Aldehyde test was strongly positive. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·5 g., (2) 0.1 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·1 g., (7) 0·15 g., (8) 0·2 g., (9) 0·2 g. and (10) 0·2 g. The number of injections was ten and the total quantity was 1·3 grms. The temperature came down to normal after the third injection. No reactions were observed during treatment. Spleen was about ½ in. below the costal arch and the general condition of the patient was very satisfactory.

CASE NO. 7.—U., M.M., æt. 25, came under treatment with history of constant fever for 8 months. Spleen

measured 7 in. and liver 2 in. below the costal margin. Aldehyde test was fairly positive. Patient was given daily intravenous injections of urea stibamine in the following doses : (1) 0·05 g., (2) 0·1 g., (3)0·1 g., (4) 0·1 g., (5) 0·1 g. (6) 0·2 g., (7) 0·2 g., (8) 0·2 g., (9) 0·2 g. and (10) 0·2 g. The number of injections was ten and the total quantity was 1·45 grms. The temperature came down to normal after the third injection. No reactions were observed during treatment. Spleen was 2 in. below the costal arch and the liver was not palpable and the general condition of the patient was very satisfactory.

CASE NO. 8.—N., M.M., æt. 10, came under treatment with history of occasional attacks of fever for about one year and with double rise of temperature for two months and a half. Spleen measured 6½ in. and liver was not palpable. Aldehyde test was fairly positive. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·5 g., (2) 0·1 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·1 g., (7) 0·1 g., (8) 0·15 g., (9) 0·15 g. and (10) 0·15 g. The number of injections was ten and the total quantity was 1·1 grms. The temperature came down to normal after the third injection and remained so during the period of observation. No reactions. Spleen was 2 in. below the costal arch and the general condition of the patient was very satisfactory.

CASE NO. 9.—A. B., A.I. M., æt. 28, came under treatment with history of remittent fever for about a month. Spleen measured 2 in. below the costal arch. Aldehyde test negative and urea stibamine test positive. Patient was given intravenous injections of urea stibamine on successive days in the following doses : (1) 0·05 g., (2) 0·05 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·2 g., (7) 0·2 g. and (8) 0·2 g. The total number of injections was eight, the total quantity being one gram. The temperature came down to normal after the second injection. No reactions. Spleen

could not be felt below the costal margin, and the general condition of the patient was very much improved.

CASE No. 10.—R. D., H.M., æt. 36, came under treatment with history of continuous fever for 13 days. There was no enlargement of the spleen. Aldehyde test negative. Widal positive against *B. typhosus* 1/100. L. D. bodies were found in the thick film from peripheral blood. Patient was given intravenous injections of urea stibamine on successive days in the following doses: (1) 0·05 g., (2) 0·05 g., (3) 0·1 g., (4) 0·1 g., (5) 0·1 g., (6) 0·2 g., (7) 0·2 g. and (8) 0·2 g. The number of injections was eight and the total quantity was one gram. The temperature came down to normal after the fourth injection. No reactions. General condition of the patient was satisfactory.

CASE No. 11.—A. K., H.F., æt. 18, came under treatment with history of irregular attacks of fever for 9 months. Spleen measured 7 in. below the costal arch. Aldehyde test strongly positive. Patient was given intravenous injections of urea stibamine on successive days in the following doses: (1) 0·05 g., (2) 0·1 g., (3) 0·1 g., (4) 0·1 g., (5) 0·15 g., (6) 0·2 g., (7) 0·2 g., (8) 0·2 g., (9) 0·2 g. and (10) 0·2 g. The number of injections was ten, the total quantity being 1·5 grms. The temperature came down to normal after the third injection. No reactions. Spleen could not be felt below the costal margin at the end of the period of observation and general condition of the patient considerably improved.

CASE No. 12.—B., H.F., æt. 72, came under treatment with history of fever with bronchitis for 6 months, and hæmoptysis on several occasions. Spleen was 6 in. below the costal arch. Aldehyde test strongly positive. Patient was given intravenous injections of urea stibamine on successive days in the following doses: (1) 0·05 g., (2) 0·05 g., (3) 0·05 g., (4) 0·05 g., (5) 0·075 g., (6) 0·075 g., (7) 0·1 g., (8) 0·15 g., (9) 0·2 g. and (10) 0·2 g. The number

of injections was ten, the total quantity being one gram. The temperature came down to normal after the eighth injection. No spleen could be felt below the costal margin and the general condition of the patient was satisfactory.

CASE NO. 13.—B. M., H.F., æt. 17, came under treatment with history of fever for 15 days with double rise and intermission and with profuse sweating. Spleen measured 2 in. below the costal arch. Aldehyde test was positive. Urea stibamine test was strongly positive. Administration of quinine produced no effect. Patient was given intravenous injections of urea stibamine on successive days in the following doses: (1) 0·05 g., (2) 0·05 g., (3) 0·05 g., (4) 0·1 g., (5) 0·1 g., (6) 0·15 g., (7) 0·15 g., (8) 0·15 g., (9) 0·2 g. and (10) 0·2 g. The number of injections was ten and the total quantity was 1·2 grms. The temperature came down to normal after the first injection. No reactions. Spleen could not be felt below the costal margin and the patient's general condition was satisfactory.

CASE NO. 14.—R., A. I. F., æt. 24, came under treatment with a history of periodical attacks of fever and epistaxis for four months Spleen measured 4 in. below the costal arch. Face was puffy, ankles swollen and patient was anæmic. Aldehyde test positive. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·05 g., (2) 0·05 g., (3) 0·05 g., (4) 0·05 g., (5) 0·1 g., (6) 0·1 g., (7) 0·1 g., (8) 0·15 g., (9) 0·15 g., (10) 0·15 g., (11) 0·15 g., (12) 0·2 g. and (13) 0·2 g. The number of injections was thirteen and the total quantity was 1·5 grms. The temperature came down to normal during treatment and remained so during the period of observation. No reactions. All the symptoms disappeared. Spleen could not be felt below the costal margin at the end of the period of observation. Patient's general condition was satisfactory.

CASE No. 15.—L., M.M., æt. 14, came under treatment with history of fever for about 6 months. There was severe cachexia and the patient could not stand on his legs. Spleen extended to the pelvis. Aldehyde test strongly positive. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·025 g., (2) 0·025 g., (3) 0·025 g., (4) 0·025 g., (5) 0·05 g., (6) 0·05 g., (7) 0·05 g., (8) 0·05 g., (9) 0·075 g., (10) 0·075 g., (11) 0·1 g., (12) 0·1 g., (13) 0·1 g., (14) 0·1 g., (15) 0·15 g. and (16) 0·2 g. The number of injections was sixteen and the total quantity was 1·2 grms. The temperature came down to normal during treatment and remained so during the period of observation. No reactions. At the end of the period of observation the spleen could not be felt at the costal margin and his general condition remarkably improved.

CASE No. 16.—S. K., M.F., æt. 38, came under treatment with history of fever for 3 months and marked anæmia. Spleen palpable on deep inspiration. Aldehyde test positive. Patient was given daily intravenous injections of urea stibamine in the following doses: (1) 0·05 g., (2) 0·1 g., (3) 0·15 g., (4) 0·15 g., (5) 0·15 g., (6) 0·15 g., (7) 0·15 g., (8) 0·2 g., (9) 0·2 g. and (10) 0·2. The number of injections was ten, the total quantity being 1·5 grms. The temperature came down to normal after the first injection. No reactions were observed during treatment. Spleen could not be felt on deep inspiration and there was well marked general improvement of the patient.

TABLE SHOWING THE NUMBER OF INJECTIONS AND THE TOTAL AMOUNT OF UREA STIBAMINE IN EACH CASE

| | Case No. | No. of injections | Total Amount of Urea Stibamine |
|---|---|---|---|
| (a) | 1 | 10 | 1·2 g. |
| | 2 | 11 | 1·5 g. |
| | 3 | 9 | 1·15 g. |
| | 4 | 12 | 1·15 g. |
| | 5 | 11 | 1·35 g. |
| | 6 | 10 | 0·95 g. |
| | 7 | 10 | 1·7 g. |
| | 8 | 10 | 1·175 g. |
| | 9 | 8 | 1·3 g. |
| | 10 | 6 | 0·55 g. |
| | 11 | 10 | 1·1 g. |
| (b) | 1 | 11 | 1·75 g. |
| | 2 | 13 | 2·5 g. |
| | 3 | 15 | 2·35 g. |
| | 4 | 13 | 1·72 g. |
| (c) | 1 | 8 | 0·85 g. |
| | 2 | 10 | 1·25 g. |
| | 3 | 4 | 0·2 g. |
| | 4 | 8 | 0·625 g. |
| | 5 | 6 | 0·715 g. |
| | 6 | 10 | 1·3 g |
| | 7 | 10 | 1·45 g. |
| | 8 | 10 | 1·1 g. |
| | 9 | 8 | 1·0 g. |
| | 10 | 8 | 1·0 g. |
| | 11 | 10 | 1·5 g. |
| | 12 | 10 | 1·0 g. |
| | 13 | 10 | 1·2 g. |
| | 14 | 13 | 1·5 g. |
| | 15 | 16 | 1·2 g. |
| | 16 | 10 | 1·5 g. |

## OBSERVATIONS

This paper describes a series of cases of kala-azar cured by the intensive treatment with urea stibamine given intravenously on successive days in the following hospitals: (1) Carmichael Medical College Hospital, Calcutta, (2) Chittaranjan Hospital, Calcutta, (3) Campbell Hospital,

Calcutta and (4) the Out-Patient Department, Chittaranjan Hospital, Calcutta. There were no untoward symptoms and temperature generally came down to normal after one or two injections. The number of injections was, on an average, about ten and the total amount of urea stibamine required in each case is shown in the above table.

We recommend that in suitable cases this intensive treatment with urea stibamine should be adopted. One remarkable feature in this form of treatment was that the total amount of urea stibamine required for a cure, in twenty-nine out of thirty-one cases, was about 1·5 grams or less. This would reduce the cost of treatment to a considerable extent. This fact is of much importance from an economic standpoint in the treatment of the disease where its mass treatment has to be taken into consideration by any Government as a prophylactic measure in endemic areas of kala-azar.

*Reference*

Brahmachari, Phanindranath and Brahmachari, Upendranath (1931). The Intensive Antimonial Treatment of Kala-azar. *Journal of Tropical Medicine and Hygiene*, Aug., 15, 1931.

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# CAMPAIGN AGAINST KALA-AZAR IN INDIA

(1) Segregation and removal.
(2) Mass treatment of infected individuals in early days of antimony treatment.
(3) Recent mass treatment with urea stibamine and its success in the campaign.
(4) Dermal Leishmanoid—a possible means of infection.
(5) Conclusions.

Compulsory evacuation of the infected areas in Assam (India) was the only available preventive measure against kala-azar before the days of antimonial treatment of the disease, and apparently successful results were obtained by Dodds Price by adopting measures of removal and segregation of infected individuals in certain tea gardens. Similar operations were adopted by the Government of Assam, in Golaghat, in 1912. It consisted of removing the patients and their families to a distance not less than 300 yards from the infected house. The infected house was burnt down. The valuable properties were disinfected and the less valuable ones destroyed, compensation being paid for their destruction. It was, however, observed that removal of the infected family alone was not sufficient to eradicate the infection from a village, as fresh cases of the disease appeared in the neighbouring houses, the year after the removal of the infected family. It was, therefore, decided to adopt measures to remove, as "contacts," the families

who lived in the immediately adjoining houses. The results were more successful, but the process had frequently to be repeated, year after year, as the disease recurred in houses beyond the excised area. In many cases, and particularly in areas of old-standing infection, it was found that better results followed by moving the whole village at the beginning, instead of moving sections of the village, in successive years. In this way, the mortality was less and the cost remained the same. At first these operations seemed to be hopeful, and in 1916 it was hoped that if no new factors arose to vitiate the calculations, one might look for the extinction of the disease in the areas treated in the above way, within a year or two. This hope was not, however, realised, as an epidemic of influenza, during the cold weather of 1918-1919, changed the whole situation, and there was a recrudescence of the disease in areas where it had been dormant. Further the disease threatened to spread to areas previously uninfected.

To control the disease the Government of Assam some time ago adopted certain measures under the provisions of the Epidemic Diseases Act. The regulations provided for the notification, on the recommendation of the Sanitary Commissioner, of any village area found to be infected with kala-azar, for the prohibition of migration from that area, and for the compulsory removal of any of its inhabitants from an infected site, and for the destruction of the infected house and property. The infected families, that is to say, those in which a case of kala-azar was discovered, were grouped in an "infected camp," and "contacts," *i.e.*, their neighbours and any other families which for any reason were under suspicion, were located in another group of houses, forming the "contact camp." The remainder were located in a "healthy camp," which was meant to form the nucleus of a village. There was no migration from notified areas, and the intercommunication with un-

infected villages was greatly limited and chiefly confined to visits between relatives. It was found that the removal of a community from an infected area and treatment of those among them who were infected with the disease would terminate the outbreak in that particular community, but the method upon a large scale was prohibitively expensive. It was found that inspite of the effective prevention of migration of individuals from infected areas and limitation of communication between infected and healthy villages, a gradual diffusion of the disease took place, and year after year, new infections were discovered in previously uninfected villages to which the same expensive measures of removal and control had to be applied. When the general recrudescence which followed the epidemic mentioned above had to be faced, experience showed that segregation on the scale which would be necessary to deal with a widespread prevalence would be prohibitively expensive and administratively impossible (McCombie Young).

Since the treatment of kala-azar by intravenous injection of tartar emetic had been introduced, treatment as a method of prevention was originally put forward by Knowles in 1920 as an alternative to the methods of prevention by segregation. The results following mass treatment with tartar emetic were found to be encouraging and early treatment of the first one or two cases seemed to control an outbreak. In some cases it appeared to extinguish the disease entirely, perhaps by preventing the establishment of the conditions of the site infection, if the first case had acquired the infection elsewhere. In practically all cases the mass treatment seemed to prevent the outbreak from assuming extensive proportions. The indications seemed to be that, where only one case came under observation, there was a reasonable chance that no more cases would occur if early treatment was adopted. When several cases were seen for the first

time, and if they had remained for some time unrecognized and untreated, and the opportunities for the establishment of site infection were ample, then no amount of treatment of cases seemed to be able to extinguish an outbreak, and under those circumstances a perennial crop of cases was to be expected. It seems that early compulsory treatment had a distinct preventive action where it was efficiently applied in the early stages of a village infection, but removal to a fresh site was still necessary to terminate an infection when it became deeply rooted by delay in action.

The present-day treatment campaign against kala-azar in Assam has been of immense value as a prophylactic measure. The present-day campaign against the disease in Assam is well described in Health Bulletin No. 9 (Government of India Central Publication Branch, 1927), containing the *Treatment Campaign against Kala-azar in Assam*, as drawn by Major Murison, Director of Public Health, Assam, and the following extracts are made therefrom :—

The treatment of the disease in Assam with tartar emetic began in 1919, when only a comparatively small number of cases were treated. In the special kala-azar dispensaries and out-centres, sodium antimony tartrate, manufactured by Messrs. Burroughs Wellcome and Company, London, and put up in "soloid" form, was formerly used exclusively.

Although treatment with this drug was very successful, it had the disadvantage of being long and tedious. Treatment was, therefore, difficult to enforce, as patients who had been completely incapacitated by the disease improved so considerably after a few injections that they discontinued treatment altogether or attended very irregularly. This irregularity made it very difficult to effect complete cures. In spite of the regulations in force under the Epidemic Diseases Act to compel patients to undergo a complete course of treatment, the campaign against the disease was

greatly handicapped by the number of patients who were stopping treatment.

To overcome this difficulty *communiqués* were regularly issued inviting the co-operation of the people. Much propaganda work has been done by means of lantern demonstrations and illustrated posters and pamphlets on the disease, emphasizing the grave dangers of stopping treatment before a complete cure has been effected. This had some effect in reducing the "stopped treatment" cases. It was felt that the above difficulties would be still further overcome, if some drug could be introduced, which was not only as efficacious as sodium antimony tartrate, but took a much shorter time to effect a cure.

In 1922 the attention of the Government of Assam was drawn to the most brilliant results obtained by Major Shortt, Director, Kala-azar Commission, in the treatment of kala-azar, while working under the auspices of the Indian Research Fund Association at the Pasteur Institute, Shillong, by the use of an aromatic antimonial discovered by me in 1921 and named *Urea Stibamine*, and which I sent to him for trial in cases of kala-azar at the request of Colonel Greig, Director of Medical Research in India (a post now defunct). I had already obtained very satisfactory results in the course of my research in the treatment of the disease (*vide* my papers on Urea Stibamine in the Treatment of Indian Kala-azar).

In 1927 the campaign against kala-azar continued with unabated vigour and with conspicuous success in Assam, according to a Government Resolution on the Health Report. Both the number of cases treated and the number of deaths decreased by slightly over 30 per cent, as compared with the previous year. All districts in which the epidemic was of importance shared in the decrease.

About the middle of this year the universal mass free treatment of the disease in Assam by the use of urea

stibamine (Brahmachari) was introduced throughout the province. This drug effects a cure in a much shorter time than the inorganic salts of antimony which were first successfully employed against the disease, and this results in a very much smaller number of cases which give up the treatment before a complete cure is effected. Cases of relapses, which are often more obstinate to treatment than original attacks, are thus reduced to a minimum.

Major Shortt, Director of the Kala-azar Commission in India, lecturing at the Health Interchange of the League of Nations, while describing the campaign against kala-azar in Assam, considered it to be one of the most successful experiments in public health measures ever adopted, and sounded a very strong note of warning against the relaxation of the effort, as the disease ceases to have epidemic, and again assumes endemic form. He emphasized that it was of the utmost importance to eradicate the endemic foci of infection, so that it might not again, after a number of years, assume an epidemic form. He stated that "we can only repeat, and ask others to repeat, that the measures taken by treatment and otherwise, within the next few years, are likely to be more important, and to have a more far-reaching effect, if resolutely pursued, than the most intensive anti-kala-azar measures carried on during the height of the epidemic year."

I would now like to point out that in adopting prophylactic measures one has to guard against possible kala-azar carriers as a source of infection. Cases of kala-azar with obscure anæmia and œdema, with or without enlargement of spleen and with history of little or no fever, may sometimes be found in endemic areas of kala-azar and these cases should be properly searched for and properly treated so as to prevent their remaining unsuspected and thereby becoming sources of infection in areas which have apparently been assumed to be completely free from the disease by mass treatment.

Still more difficult are cases of kala-azar previously treated with antimony and apparently cured and which, after one or two years, manifest a remarkable granuloma in the skin containing *Leishmania donovani*, a condition first discovered by me and named by me as "Dermal Leishmanoid." These cases are not so uncommon as I first believed them to be. That being the case, I would suggest that such propaganda should be arranged by the Health Department of infected areas that patients cured of kala-azar might be educated to have their skin carefully examined for the presence of Dermal Leishmanoid for one year or so after completion of treatment. If such lesions are discovered, they should again be treated with a course of urea stibamine.

Whatever may be discovered in future about the actual mode of infection, these skin lesions are always infective as they give positive flagellate culture on N.N.N. medium. They may propagate the disease by direct contact with the abraded skin of an healthy individual, or if the sandflies are responsible for the propagation of the disease then, as has been recently pointed out by Wenyon, it must be a relatively easy matter for them to take up the parasites from these lesions in the skin as they do in oriental sore.

The following extract from the speech of His Excellency Sir John Kerr, while bidding farewell to the second Legislative Council in Assam (1926), shows the value of the campaign against kala-azar in Assam. After referring to the value of the treatment of this disease with urea stibamine, His Excellency said: "We may now say that victory, if not in sight, is assured. The progress in the campaign against kala-azar in Assam has been phenomenally rapid, and if it continues at the present rate, there is an excellent prospect of the dread scourge being brought under complete control in a few years."

I do not desire to make here any speculative suggestions for the destruction of, or protection against, sandflies

as a prophylatic measure, since they may be as valueless as any that could have been formulated a few years ago for the destruction of bed-bugs, which were once supposed to be the carriers of the disease. Instead of asking the poor patient to adopt measures which may be expensive for him to carry out, and subsequently turn out to be ineffective one would like to wait till the transmission problem is solved. I would now like to listen with very keen interest to any measures that may have been adopted for the eradication of kala-azar in the Mediterranean Basin.

In conclusion, I would point out that General Gorgas, speaking in 1914 on yellow fever control in the Americas, stated that its eradication would command the attention and the gratitude of the world and that the thing could be done. To-day yellow fever is in full retreat in the Americas. The same will one day be said of kala-azar, and it may be hoped that before long the disease will be completely banished from India and other parts of the world where it occurs. As I have stated in this paper, the signs of its retreat in Assam are already within sight, thanks to the intensive mass treatment of the disease with urea stibamine. When the disease disappears from the world, then one of the highest triumphs of tropical medicine will be achieved.

In my opinion, the eradication of kala-azar with the recent improvements in its treatment will be a much easier problem than that of malaria.

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# CHEMOTHERAPY OF ANTIMONIAL COMPOUNDS

In studying trypanosomiasis, Ehrlich demonstrated that the trypanosomes assimilated the organic derivatives of arsenic only when the arsenic was present in the trivalent and not in the pentavalent form. Similarly, the experiments of Kolle, Hartoch, Rothermundt and Schurmann have demonstrated that compounds containing pentavalent antimony are not organo-tropic except in large doses and are at the same time slightly parasito-tropic. Preparations containing trivalent antimony were as a rule exceedingly toxic to the organism and at the same time it was also demonstrated by the researches of these observers that for antimony compounds, soluble or insoluble, organic or inorganic, to be of therapeutic value in trypanosomiasis, the antimony must be in the trivalent form.

My more recent researches and those of others who have followed me have proved that the pentavalent aromatic antimonials are more potent in the treatment of kala-azar than the trivalent antimonyl tartrates.

Metallic antimony is also singular in its chemotherapeutic properties, having the property of being introduced into the veins in the crude form of a fine suspension without any danger of capillary blocking, as has been demonstrated by the observations of Plimmer and Fry as well as those of Ranken in trypanosomiasis and of myself in kala-azar.

I shall try to explain what I consider the mechanism by which metallic antimony exerts its parasiticidal properties.

Being an element it does not contain any group or radicals which may complicate any explanation that may be suggested for its action.

Levaditi has propounded a general law with reference to all the members of the nitrogen family of elements, occupying Group V of Mendeléeff's Periodic Table, such as arsenic antimony or bismuth. According to this law, these elements exhibit their parasiticidal properties only after they are acted upon by the tissues, *e.g.*, if fresh extract of liver is added to them, they become actively treponemicidal.

Recent unpublished experiments carried on by myself have demonstrated that soon after an intravenous injection of metallic antimony into rats in non-toxic doses it disappears from the circulation and appears mostly in the spleen and bone-marrow. In the spleen, particles of antimony can be found chiefly inside large cells with small nuclei and faintly stained protoplasm. These are probably of the nature of clasmatocyte cells.

It has been observed by Meleney and others that in kala-azar, these clasmatocyte cells are developed as a tissue reaction out of the reticulo-endothelial system. Inside these cells I hold that the antimony is converted into soluble compound containing $-Sb=O$ in a reactive state, which exerts its parasiticidal action.

The therapeutic action of metallic antimony is greater than that of $Sb_2O_3$ as well as the antimonyl tartrates, because I hold that although these compounds contain the radicle $-Sb=O$, yet it does not exist in them in the reactive stage upon which depends the therapeutic value of an antimonial.

The mechanism of the action of metallic antimony when introduced into the vein, is therefore that it is first taken up by the cells of the reticulo-endothelial system and then converted into a soluble antimony compound containing $-Sb=O$ in the reactive stage (stiboxyl).

By studying the excretion of antimony in man after intravenous injections of a therapeutic antimonial, I have observed that in the case of tartar emetic, the curve of excretion is one slowly converging to the base line. Pentavalent aromatic antimonials of the type of urea stibamine follow a curve, the first portion of which, representing the excretion during the first 24 hours, is abrupt, similar to what is observed in the case of organic arsenicals, and the second portion follows a curve similar to that found in the case of tartar emetic in which the antimony exists in the trivalent form. In other words, after 24 hours or so the organic pentavalent antimonial undergoes a reduction giving rise to a compound, $-Sb=O$, in the reactive stage. This also explains why urea stibamine is more efficacious than either tartar emetic or $Sb_2O_3$. On the other hand, inorganic antimonates are more or less useless therapeutically as they are excreted as antimonates in the urine without undergoing any change in the body.

Generally speaking, the toxicity of the antimonyl tartrates depends upon their antimony content. Notable exceptions are in the case of ammonium antimonyl tartrate and quinine antimonyl tartrate, in which the toxicity is low, especially in the latter. The possibility of using these compounds in therapeutics should therefore be borne in mind. The latter may have the advantage of combining the therapeutic properties of antimony and quinine. Ammonium antimonyl tartrate is the least toxic of all the inorganic tartrates and therapeutically I consider it superior to sodium antimonyl tartrate or tartar emetic.

I have not been able to confirm the observations of Farghar and Gray that the toxicity of the antimony content of quinine antimonyl tartrate is only one-fifth that of tartar emetic, though I agree with them that its toxicity is less than that of tartar emetic. I have confirmed their observations that quinine acid tartrate, on boiling with antimony trioxide,

is converted into the more toxic quino-toxin antimonyl tartrate. I have not been able to confirm their conclusions that the sodium salt is less toxic than the potassium salt. I have confirmed Plimmer and Thompson's observations that the lithium salt is more toxic than the sodium or potassium salt and that the toxicity of sodium and potassium salts is equal.

Regarding the excretion of antimony, I and my collaborators have observed a law that after single or repeated injections of an antimonial the amount of antimony excreted in the urine during the first twenty-four hours is fairly proportional to the amount injected.

Further there is a concentrative limit of antimony in the body after repeated injections of tartar emetic and this is the safeguard against any cumulation of this drug in the tissues when the concentration reaches this point.

*Aromatic Antimonials.*—Antimonials of the stibeno-benzene type have not yet come into use in the treatment of human diseases, though they have been used with indifferent results in the case of certain diseases of animals.

*Phenyl-stibinate of Sodium.*—The minimum lethal dose of phenyl-stibinate of sodium is three and a half times less than that of *p*-amino-phenyl-stibinate of sodium, while its maximum tolerated dose is 35 times less. Injected into lower animals, it gives rise to a hæmorrhagic nephritis and other symptoms of severe antimony poisoning. This compound has little or no use in therapeutics, but the introduction of $NH_2$ into its benzene nucleus at once diminishes its toxicity and raises its therapeutic value to a remarkable extent.

*Acetyl-para-amino-phenyl-stibinate of Sodium.*—By acetylation, it is expected on theoretical grounds that the toxicity of *p*-stibanilic acid would be reduced. This acetyl compound has been used in the treatment of kala-azar with unsatisfactory results. Besides, it becomes toxic with age in

India and it has now come into disuse. But I still hold that pure acetyl-*p*-amino-phenyl-stibinate of sodium should again be given a trial in kala-azar and may in future be found to be free from all those toxic effects that were exhibited in the original compound put on the market under the name of stibenyl.

*Stibamine.*—As the sodium salt formed after hydrolysis of the acetyl compound corresponds to atoxyl or soamin and is sodium-*p*-stibanilate, I have named it stibamine. Comparing its toxicity with that of the acetyl compound, it will be seen that the introduction of the acetyl group into it does reduce its toxicity as in the case of corresponding arsenic compound. Thus, while in the case of ars-acetin the toxicity is markedly diminished by the introduction of acetyl group into atoxyl (being one-fifth that of atoxyl), in the case of sodium-*p*-stibanilate and the acetyl compound, my observations have shown that their toxicity is the same, the M.T.D. being ·35 grammes per kilo of body weight in guinea-pigs given intramuscularly in the case of both the compounds.

*Chloro-acetyl-para-amino-phenyl-stibinate of Sodium.*—This is a compound formed by the replacement of one hydrogen atom in the benzene nucleus of the acetyl compound by chlorine. I have given it the name of chloro-stibacetin. It has been put on the market under the name of *stibosan* (von Heyden) and it has been claimed that the introduction of chlorine increases its stability and that it can be stored in ordinary stoppered bottles and weighed out when required and is therefore most useful for general purposes. In my opinion, such a compound has more or less the same stability as the inorganic antimonates and, therefore, there is less chance of the production of the reactive $-Sb=O$ in the tissues after their administration which I consider responsible for the beneficial results following the administration of an antimony compound.

*Urea stibamine.*—I consider that urea stibamine owes its therapeutic value to the presence of $NH_2CO$. The same holds good with the compound which I have named *stib-glycine-amide.*

If my theory is correct that the therapeutic value of an inorganic antimonial depends upon the ease with which it can be converted in the tissue into an antimony compound cotaining a radicle $-Sb=O$ in the reactive stage, then the same should also apply to the aromatic antimonials.

I have given above certain aspects of chemotherapy of antimony in kala-azar in respect of which it has been mostly studied. If the principle that I have suggested regarding the chemotherapy of antimonial compounds in kala-azar infection does not apply to other diseases in which antimony is indicated, then the problem must be much more complicated than what one would at first imagine. I would, therefore, like to hear from my audience here whether the same principle that I have propounded above applies to other diseases as they occur in Egypt, such as bilharziasis. I have no experience of the treatment of this disease and I would like to listen with very keen interest to the experience of the learned speakers here regarding the chemotherapy of antimony with regard to this disease as well as trypanosomiasis.

We are just beginning to reach the fringes of the science of chemotherapy in general and this especially holds good in the case of that wonderful element antimony.

Some of the compounds referred to here have been mentioned in my paper on "*Urea Stibamine in the treatment of Indian Kala-azar*" which is to be read at this Congress.

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# UREA STIBAMINE IN THE TREATMENT OF INDIAN KALA-AZAR

My justification in reading this paper is to bring to the notice of the medical profession in Egypt and other parts of the Mediterranean basin the value of an antimony compound which has been of immense benefit in the treatment of Indian kala-azar. I shall begin my remarks by giving here a short history of its introduction in the treatment of kala-azar.

The chemotherapy of aromatic antimonials in kala-azar infection has been the subject of my research for many years. In 1920, shortly after I had been financed by the Indian Research Fund Association for carrying on researches into the treatment of kala-azar, *acetyl-p-amino-phenyl stibinate of sodium* and *amino-phenyl-stibinate of sodium* were prepared for the first time in India in my laboratory in the Calcutta Campbell Medical School, and I immediately brought to the notice of Government, the Governing Body of the Indian Research Fund Association, and the then Secretary of the Calcutta School of Tropical Medicine, the possibilities of the potentialities of these compounds in the treatment of Indian kala-azar, my conclusions being based on the theoretical grounds, from an analogy of the value of the corresponding compounds of arsenic, namely, *ars-acetin* and *atoxyl* in the treatment of certain protozoal diseases.

The acetyl compound (=stibacetin, stibenyl) was used more or less successfully outside India in the treatment of kala-azar and other forms of Leishmaniasis (Caronia,

Kharina-Marinuchi, Spagnoloi). Manson-Bahr successfully used it in a case of kala-azar. In India Mackie and others found unsatisfactory results in the treatment of kala-azar with stibenyl. Early in 1921 I discovered that urea could combine with stibanilic acid and that the resulting compound surpassed all my expectations in its value in the treatment of Indian kala-azar. Its introduction and my researches into the chemotherapy of antimonial compounds in kala-azar infection under the auspices of the Indian Research Fund Association opened up a new vista in the treatment of the disease in India, in face of the untoward results obtained with stibenyl by Mackie and others. This compound I named *urea stibamine*.

Until recently tartar emetic or sodium antimonyl tartrate was extensively used for the treatment of kala-azar in India. These have now been completely replaced by urea stibamine, on account of its high therapeutic value and marked superiority over the antimonyl tartrates.

So remarkable was the therapeutic value of this compound that even before the results of my observations were published the medical profession in and outside Calcutta came to recognize its value, soon after its discovery, from the reports of cases treated with the drug in my wards in the Campbell Hospital, Calcutta. My first series of successful cases were published in October, 1922. In 1923, Shortt and Sen in Assam, reported having obtained more brilliant results with this compound than what was obtained by me in my first series of cases.

The Governing Body of the Indian Research Fund Association quickly recognized its value from the reports of my cases in Calcutta and also of those obtained from Shortt and other successive Directors of the Pasteur Institute, Shillong, from Christophers, Director of Kala-azar Commission, who reported from his experience in Assam about its remarkable efficacy, from medical officers of tea estates in

Assam, and from the Government of Assam. In Calcutta its value was recognized by the physicians of the Calcutta Medical College Hospitals and successive Superintendents of the Calcutta European Presidency General Hospital. In these institutions it was quickly introduced and extensively used with brilliant results. Its reputation quickly spread all over Assam, Bengal, Bihar and Orissa, and to more distant places in India such as Madras, Sanawar, Simla Hills and other places too numerous to mention, and every observer who used the drug was convinced of the great advance made by the discovery of the compound and its introduction in the treatment of Indian kala-azar.

Remarking on its therapeutic value, the Secretary, Scientific Advisory Board, Indian Research Fund Association, wrote as follows: "Both the Director of the Kala-azar Commission and Lieut.-Col. E. D. W. Greig consider that this drug has a highly specific action in kala-azar, and its value has been abundantly testified to by those who tried it both in so far as it shortens the period of treatment and in so far as it seems able to cure intractable cases or cases which are resisting the antimony treatment."

I give here some of the important points with regard to the compound.

*Chemical Constitution and Toxicity.*—I consider that the chemical constitution of urea stibamine is $NH_2.CO.C_6H_5.SbO.OH.ONH_4$ containing the group $NH_2.CO$.

Table I.

| Dose per kg. | Number of guinea-pigs used. | Number died | Remarks |
|---|---|---|---|
| 0·7 grm. | 4 | 4 | M. L. D. |
| 0·65 ,, | 3 | 2 | Maj. L. D. |
| 0.6 ,, | 4 | 2 | — |
| 0·5 ,, | 2 | 1 | — |
| 0·45 ,, | 4 | 1 | — |
| 0·4 ,, | 4 | 1 | — |
| 0·35 ,, | 4 | Nil | M. T. D. |

The maximum tolerated dose of urea stibamine per kilogramme of body weight is twenty-three times that of tartar emetic in the case of the guinea-pig. The effective dose of urea stibamine in the treatment of kala-azar is five-sevenths the tolerated dose for the guinea-pig, while in the case of tartar emetic it is eight times the tolerated dose for the same animal. Urea stibamine is, therefore, a much safer antimonial for use in the treatment of kala-azar than tartar emetic or other antimonyl tartrates.

Table II.—The value of urea stibamine in the early stage of the disease.

| | Cal.Med. Coll. Hosp.—Barnardo, McCay and author | | | | | | Pasteur Institute, Shillong. Shortt, Greig and Kundu | | | | | Hantley Tea Estates.—Foster and Banerjee. | | Authoi and Maity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 1 | 2 | 3 |
| Duration of illness in days .. | 18 | 4 | 120 | 8 | 10 | 21 | 135 | 90 | 120 | 60 | 90 | 7 | 15 | 90 | 150 | 150 |
| Amount in gm. after which cult. neg. .. | 0·35 | 0·3 | 0·4 | 0·4 | 0·3 | 0·2 | - | - | 0·7 | 0·7 | 0·95 | — | — | 0·5 | 0·2 | 0·2 |
| Total amount in grm. given .. | 0·35 | 0·3 | 0·4 | 0·5 | 0·5 | 0·2 | 0·7 | 0·75 | 0·7 | 0·7 | 0·95 | 0·35 | 0·3 | 0·5 | 0·2 | 0·35 |
| Period of treatment (in days) . | 14 | 10 | 12 | 10 | 14 | 5 | 7 | 7 | 5 | 5 | 5 | — | 7 | 4 | 5 | 5 |

It will be seen from the above that urea stibamine cuts short the course of Indian kala-azar to a remarkable degree if administered in its early stage.

Table III.—A series of cases cured with urea stibamine some of which had an intensive course of treatment consisting of multiple injections every 24 hours.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Duration of illness (in months) | 3 | 5 | 5 | 7 | 2 | 3 | 1 | 5 | — | — | — |
| Amount in grms. after which blood culture neg. ... | 0·15 | 0·85 | 0·3 | 0·65 | 0·65 | 0·8 | 0·4 | 0·4 | 0·75 | 1·4 | 0·65 |
| Total amount in grms. injected ... ... ... | 0·35 | 0·35 | 0·3 | 0·75 | 0·85 | 0·9 | 0·4 | 0·4 | 1·35 | 1.2 | 1·15 |
| Period of treatment (in hours) | 120 | 72 | 72 | 36 | 58 | 60 | 54 | 32 | 168 | 288 | 192 |
| Days of observation after treatment ... ... | 36 | 25 | 15 | 25 | 6 | 12 | 18 | 33 | 40 | 55 | 210 |

Table IV.—A number of cases of Indian kala-azar cured by less than one gramme of urea stibamine.

| | Cal. Med. Coll. Hosp.—Barnardo, McCay and author | | | | | Pasteur Inst. Shillong,—Shortt, Greig & Kundu | | Author and Maity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Duration of illness (in days) ... ... | 60 | 90 | 10 | 21 | 21 | 240 | mths. 17 | 90 | — | — | — |
| Amount in grm. after which cult. neg. ... | 0·55 | 0·65 | 0·55 | 0·9 | 0·4 | — | 0·95 | 0·7 | 0·7 | 0·5 | 0·6 |
| Total amount (in grm.) ... | 0.55 | 0·8 | 0·7 | 0·9 | 0·55 | 0·65 | 0·95 | 0·7 | 0·7 | 0·7 | 0·6 |
| Period of treatment (in days) | 15 | 14 | 10 | 22 | 14 | 6 | 7 | 5 | 6 | 9 | 5 |

In a combined series of 325 published cases, 98·47 per cent of the cases were cured. One of the cases died of extreme asthenia, being admitted at the age of 65 in a moribund condition. In 298 of these cases, proof of cure was microscopic and cultural examination and disappearance of symptoms, and in twenty-seven cases proof of cure was clinical disappearance of the symptoms and subsequent observation of the cases. One case was resistant.

*Advantages of treatment with Urea Stibamine.*—(1) The short course occupying only two to three weeks for a complete cure. (2) The appearance of early and marked leucocytosis, rapid disappearance of the fever, of splenic and hepatic enlargement and of anæmia, œdema and cachexia, in fact, of all the symptoms of the disease, and reversion to normal state of health. (3) The absence of symptoms of intolerance after its administration. (4) It is most valuable in the treatment of relapses or in the cases resistant to sodium antimonyl tartrate or tartar emetic. (5) Observations have shown that early cases may be cured after four or five injections.

*Indications and Contra-indications in the treatment of Kala-azar with Urea Stibamine.*—(1) Urea stibamine is indicated in all cases of kala-azar. It has been used in cases

complicated with bronchitis or dysentery without any untoward results. In cases of *Leishmania* dysentery it cures the condition. If, however, dysentery develops during treatment, then it is desirable to stop the injection for a time or give it in smaller doses. (2) In cases with marked nephritis and œdema, begin with smaller doses. If the œdema increases, give the doses at longer intervals than before. (3) Very advanced cases should be treated by beginning with small doses which should be slowly increased.

Urea stibamine has been observed to manifest no deterioration or other changes, either in physical and chemical characters or in therapeutic properties, if kept in sealed ampoules under ordinary conditions.

Among the other therapeutic antimonials that have been discovered by me for use in the treatment of Indian kala-azar in the course of my research are (1) stibamine, (2) chlorostibacetin, (3) stibglycine-amide (=N-phenyl-glycine-amide-*p*-stibinate of sodium), (4) the glucose derivatives of these compounds and (5) stib-hectine.

I shall not refer to any of these compounds besides pointing out that some of them have been found to be of therapeutic value in the treatment of kala-azar especially stibglycine-amide.

In 1921, while discussing with the Director of the Calcutta School of Tropical Medicine about the therapeutic value of urea stibamine, soon after its discovery, I drew his attention to the possibility of obtaining therapeutic aromatic antimonials from the "Chemische Fabrik von Heyden." Stibosan and neo-stibosan are among von Heyden's preparations that have since been used in the treatment of Indian kala-azar.

Of all aromatic antimonials that have been used up to the present time in India, the most extensive trial has been given to urea stibamine. The published reports consist of cases treated by different observers, and under different conditions,

and are therefore most valuable; it has stood the test of time for upwards of seven years.

A review of the treatment of Indian kala-azar with urea stibamine is given in my *Treatise on Kala-azar* (John Bale, Sons and Danielsson Ltd., London, 1928), to which I would refer my audience for a detailed account of the compound.

I would now quote the remarks of Shortt, Director of Kala-azar Commission, and Sen (1925) regarding the therapeutic value of urea stibamine: "We consider that the value of urea stibamine has been established as the most efficient drug at present in use in the treatment of Indian kala-azar." This statement remains equally true to-day. To this may be added the remarks of Dodds Price: "I am of opinion that urea stibamine is a most valuable remedy in the resistant types of the disease, and I strongly urge that it should be resorted to if, after a few injections of sodium antimonyl tartrate, a patient does not show marked improvement."

Gentlemen, antimony is a wonderful element. Centuries ago, Valentine, who wrote about it in *The Triumphant Chariot of Antimony*, did so with awed devotion. He said: "He who writes of antimony needs a great consideration and a most ample mind. One man's life is too short to be perfectly acquainted with all its mysteries." Centuries later, it was supposed to do so much harm that the graduates in Medicine in the University of Heidelberg had to swear never to use antimony. And to-day it has been found to be a specific in one of the most terrible of tropical diseases.

[*Reprinted from the Proceedings of the Twenty-fifth Indian Science Congress* 1938, *Part II, pp.* 285-302]

# THE CONQUEST OF KALA-AZAR

[This is ths first part of the author's Presidential Address, Section of Medical Research, Twenty-fifth Session of the Indian Science Congress, Calcutta, 1938. The second part on *Certain Observations on the Chemotherapy of Malaria* is published in the Second Volume.]

LADIES AND GENTLEMEN,

We are meeting under the most tragic circumstances. The sudden death of Lord Rutherford who was to be our General President has cast the deepest gloom over our Congress. The world has lost in him one of its most distinguished scientists who smashed the atoms, and though he breathes no more, his discoveries may one day enable one to travel to that mysterious region among the atoms and molecules wherein enters the breath of life. We express our deepest sense of sorrow to Lady Rutherford and the bereaved family. We are fortunate in having another most famous scientist as our General President and we have every reason to believe that under the guidance of Sir James Jeans, this momentous meeting of the Indian Science Congress will come to a successful issue.

The earliest epidemic of kala-azar in Bengal occurred in the seventies of the last century, when it was probably complicated with malaria. As I stated elsewhere, in this epidemic it was noted by a contemporary writer that countries that once smiled with peace, health and prosperity had been turned into hot beds of disease, misery and death, and that the fell disease had mocked every human effort, and absorbed

in its powerful grasp, day by day and inch by inch, every blessed spot which once used to be prized for its salubrity. This was the old Burdwan fever.

In more recent times the epidemic of the disease in Nowgong district of Assam produced such an appalling mortality that there was a decrease of 31·5 per cent in the population of the place in the decade 1891-1900.

The mortality from the disease has now been reduced from 90% or more to 1 or 2 per cent. Including complicated cases, it has been reduced from 99 to less than 10 per cent.

The conquest of kala-azar may be said to have begun when Cristina and Caronia obtained remarkable results in infantile kala-azar of the Mediterranean basin by the use of tartar emetic. Rogers introduced this drug into India for the treatment of Indian kala-azar and obtained similarly satisfactory results. Soon after the introduction of tartar emetic the speaker introduced sodium antimonyl tartrate for its treatment. This was taken up by others, as the compound was considered to be more powerful and less toxic than tartar emetic.

The next step in the treatment of the disease was the introduction by the speaker of intravenous injection of metallic antimony in a state of fine subdivision as an impalpable powder.

It has been observed (Brahmachari and co-workers) that when metallic antimony is injected intravenously in a state of fine subdivision, the particles are picked up by the same cells in the spleen as those that harbour the parasites of kala-azar and that in the struggle that ensues the fight ends most remarkably in the complete destruction of the parasites in the speediest way. (See Plate facing page 226)

A special outfit has been devised for the intravenous injection of metallic antimony.

The advantage of intravenous injection of metallic antimony is that the number of injections generally required is not more than three or four to bring about a complete cure.

It is one of the most powerful leishmanocides and may be tried in cases in which other antimonials have failed. The great objection to its use is the complicated technique of the operation of injection which is a serious obstacle in the mass treatment of the disease.

Although treatment with tartar emetic or sodium antimonyl tartrate was very successful in the treatment of kala-azar it was found that in the campaign against the disease it had the disadvantage of being long and tedious. In Assam which was once the hot-bed of the disease, treatment was therefore found difficult to enforce as patients discontinued treatment altogether or attended very irregularly after a few injections. This irregularity made it very difficult to effect a complete cure. The Director of Public Health, Assam, once noted that in spite of the regulations in force under the Epidemic Diseases Act to compel patients to undergo a complete course of treatment, the campaign against the disease was greatly handicapped by the large number of patients who stopped treatment. To overcome this difficulty *communiqués* were regularly issued inviting the co-operation of the people. Much propaganda work was done by means of lantern demonstrations and illustrated posters and pamphlets on the disease, emphasising the great dangers of stopping treatment before a complete cure was effected. Though this had some effect in reducing the 'Stopped Treatment' cases, still such cases continued to exist and it was felt that the difficulties in reducing the number of such cases would be overcome more effectively, if some drug could be introduced, which would be more efficacious than tartar emetic and take a much shorter time to effect a cure.

The introduction of certain organic compounds of antimony in the treatment of kala-azar infection has been the subject of the speaker's research for many years, and in 1920 some of them were prepared for the first time in India in his

laboratory in the Calcutta Campbell Hospital, and he wrote to the Indian Research Fund Association that the potentialities of the preparation of these compounds in India would in future be as great as those of cinchona plantation.

Early in 1921, the speaker discovered an urea antimony compound for the treatment of kala-azar. Its introduction and his other researches on antimonial compounds opened up a new vista in the treatment of the disease by means of therapeutic organic antimonials, just as the discovery of salvarsan led to the introduction of organic arsenicals in the treatment of spirochætal diseases. This urea compound was named 'urea stibamine.' Soon after its discovery the author suggested to the Director of the Calcutta School of Tropical Medicine the possibility of obtaining therapeutic antimonials from Von Heyden and this led to their introduction into this institution for the treatment of kala-azar.

The first series of cases treated with urea stibamine were published early in 1922. Soon after this, remarkable results were obtained with it by Shortt in Shillong to whom the compound was sent for trial at the instance of the Director of Medical Research appointed by the Government of India. The value of this compound was quickly recognized and it was introduced, after an experimental trial, by the Government of Assam for the mass treatment of kala-azar.

I now proceed to demonstrate the value of urea stibamine in the campaign against kala-azar in Assam as obtained from statistics from the Annual Public Health Reports of the Government of Assam for the years 1925-35 and the Government Resolutions thereon. This drug has been more or less successively in use by the Government of Assam for over twelve years and to-day it is in universal use in the province. For some time experiments with neostibosan were conducted side by side with urea stibamine. The use of neostibosan was subsequently discontinued.

In their resolution on the Annual Public Health Reports

of the Province of Assam for the year 1926, the Government of Assam noted that the treatment with urea stibamine proved very successful and there was a very satisfactory decrease of over 1,000 in the number of 'Stopped Treatment' cases.

Figure 1 gives the number of cases of kala-azar treated in Assam as a whole from 1925 to 1935 showing a marked fall in the incidence of the disease from 60,940 in 1925 to 11,100 in 1935. Figure 2 gives the death rate from kala-azar in Assam as a whole from the year 1925 to 1935 showing a marked fall from 6,365 in 1925 to 845 in 1935. Figure 3 gives the number of cases of kala-azar treated in the district of *Sylhet* during the same years showing a marked fall in the incidence of the disease from 10,934 in 1925 to 3,869 in 1935. Figure 4 gives the death rate from the disease in the district of *Sylhet* from the year 1925 to 1935 showing a marked fall from 2,109 in 1925 to 260 in 1935. Figure 5 gives the number of cases of kala-azar treated in the district of *Goalpara* during the same years showing a marked fall in the incidence of the disease from 6,003 in 1925 to 1,245 in 1935. Figure 6 gives the death rate from the disease during the same years in *Goalpara* showing a marked fall from 453 in 1925 to 100 in 1935. Figure 7 gives the number of cases of the disease treated in the district of *Kamrup* during the same years showing a marked fall in the incidence of the disease from 8,753 in 1925 to 1,465 in 1935. Figure 8 gives the death rate for the same years in the district of *Kamrup* from the year 1925 to 1935 showing a marked fall from 1,120 in 1925 to 176 in 1935. Figure 9 gives the number of cases treated in the district of *Darrang* during the same years showing a marked fall in the incidence of the disease from 5,262 in 1925 to 738 in 1935. Figure 10 gives the death rate for the same years in the district of *Darrang* for the years 1925 to 1935 showing a marked fall from 478 in 1925 to 91 in 1935. Figure 11 gives the number of cases

treated in the district of *Nowgong* during the same years showing a marked fall in the incidence of the disease from 13,895 in 1925 to 1,651 in 1935. Figure 12 gives the death rate from the disease for the same years in the district of *Nowgong* showing a marked fall from 1,445 in 1925 to 52 in 1935, Figure 13 gives the number of cases treated in the *Garo Hills* during the same years showing a marked fall in the incidence of the disease from 1,952 in 1925 to 690 in 1935. Figure 14 gives the death rate from the disease for the same years in the *Garo Hills* showing a marked fall from 435 in 1925 to 58 in 1935.

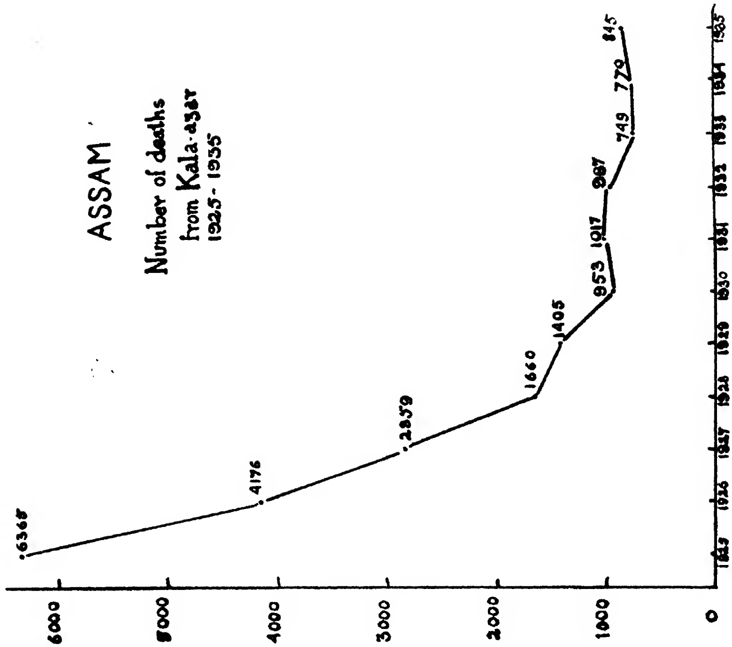
ASSAM
Number of deaths from Kala-azar 1925-1935
6368
4176
2859
1660
1405
953
1017
967
749
729
845
6000
5000
4000
3000
2000
1000
0

FIG. 2.

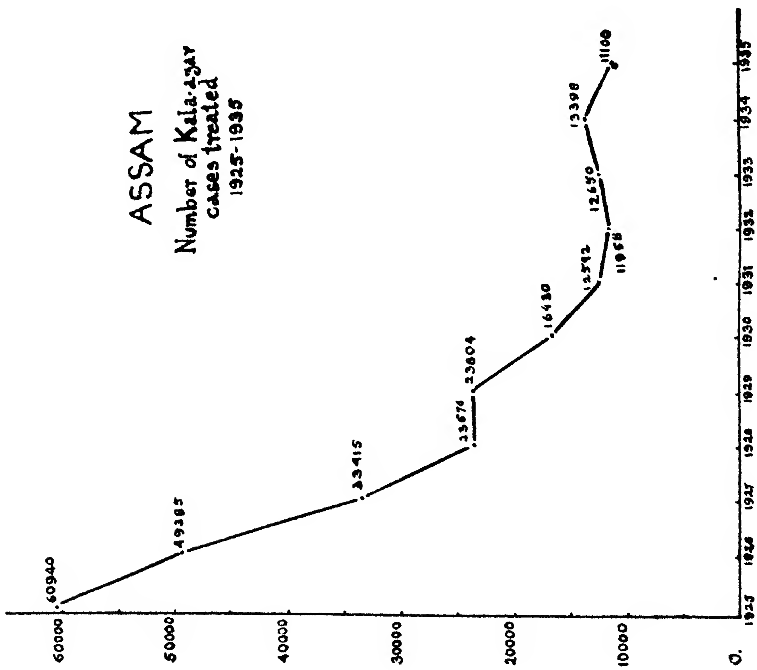
ASSAM
Number of Kala-azar cases treated 1925-1935
60940
49385
33415
23674
23804
16430
12542
11958
12650
13398
11100
60000
50000
40000
30000
20000
10000
0
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935

FIG. 1.

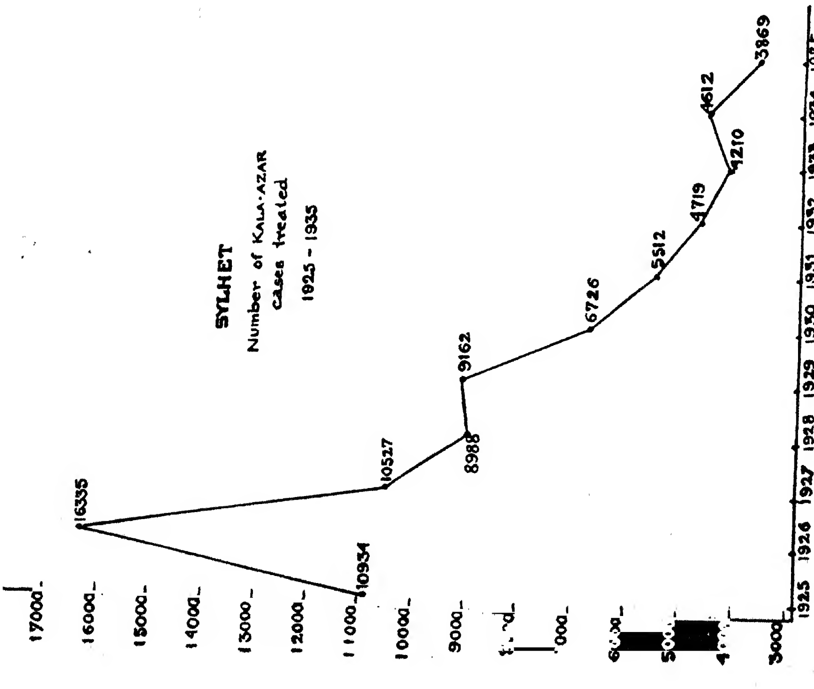
SYLHET
Number of KALA-AZAR cases treated
1925 - 1935
10934
16335
10527
8988
9162
6726
5512
4719
4210
4612
3869

FIG. 3.

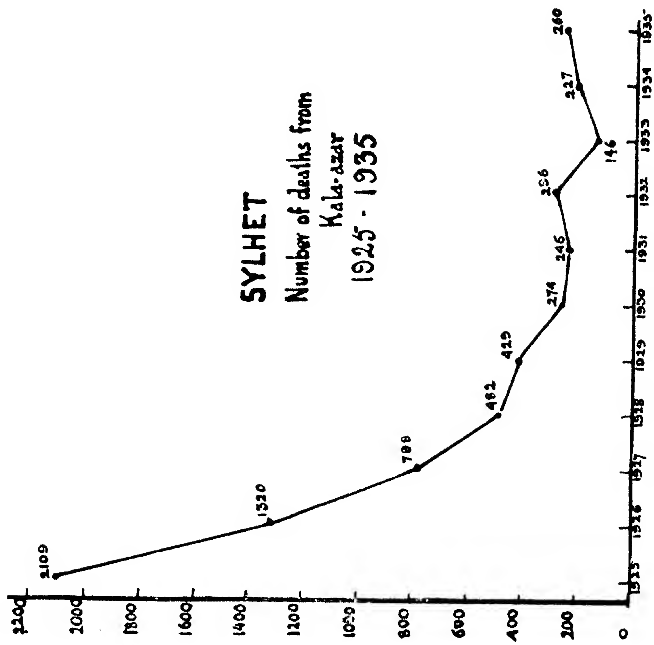
SYLHET
Number of deaths from Kala-azar
1925 - 1935
2109
1320
798
482
429
274
246
256
146
227
260

FIG. 4.

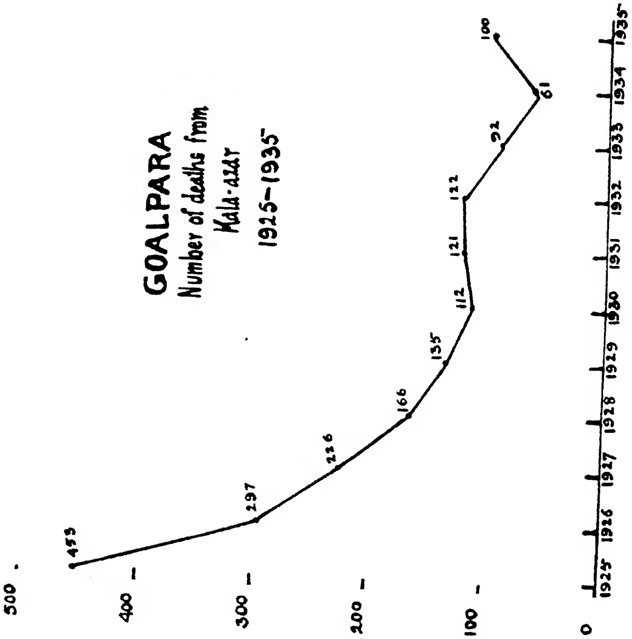
GOALPARA
Number of deaths from
Kala-azar
1925–1935
500
400
300
200
100
0
455
297
236
166
135
112
121
122
92
61
100
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935

Fig. 6.

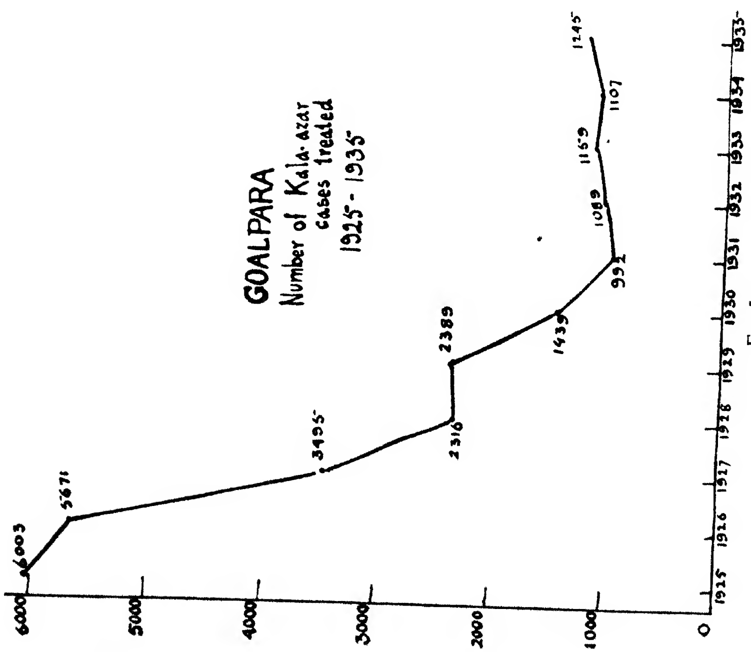
GOALPARA
Number of Kala-azar
cases treated
1925 - 1935
6000
5000
4000
3000
2000
1000
0
6003
5671
3495
2316
2389
1439
992
1089
1159
1107
1245
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935

Fig. 5.

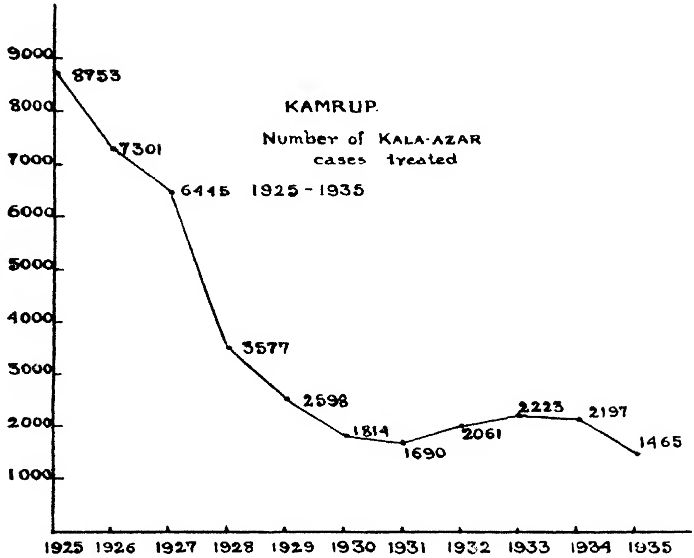
KAMRUP
Number of KALA-AZAR cases treated
1925 - 1935
9000
8000
7000
6000
5000
4000
3000
2000
1000
8753
7301
6445
3577
2598
1814
1690
2061
2223
2197
1465
1925 1926 1927 1928 1929 1930 1931 1932 1933 1934 1935

FIG. 7.

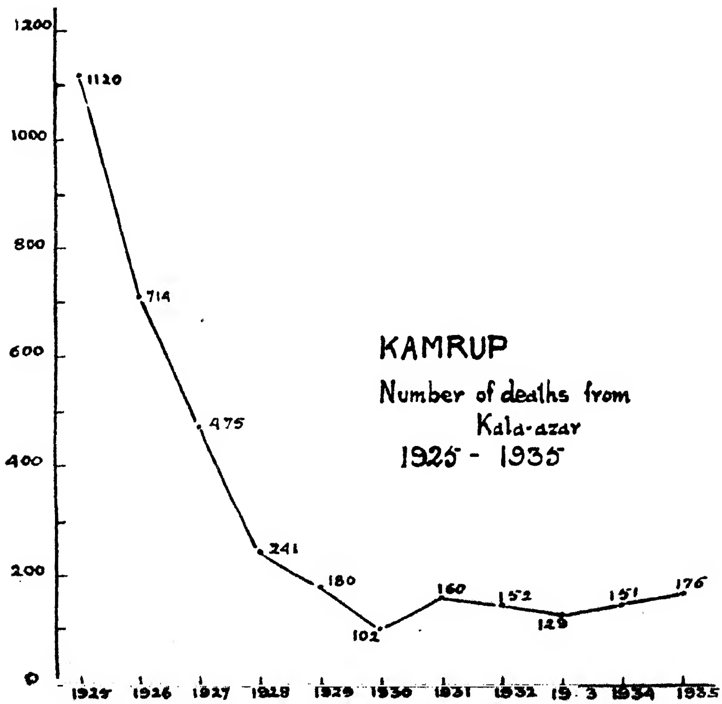
KAMRUP
Number of deaths from Kala-azar
1925 - 1935
1200
1000
800
600
400
200
0
1120
714
475
241
180
102
160
152
129
151
176

FIG. 8

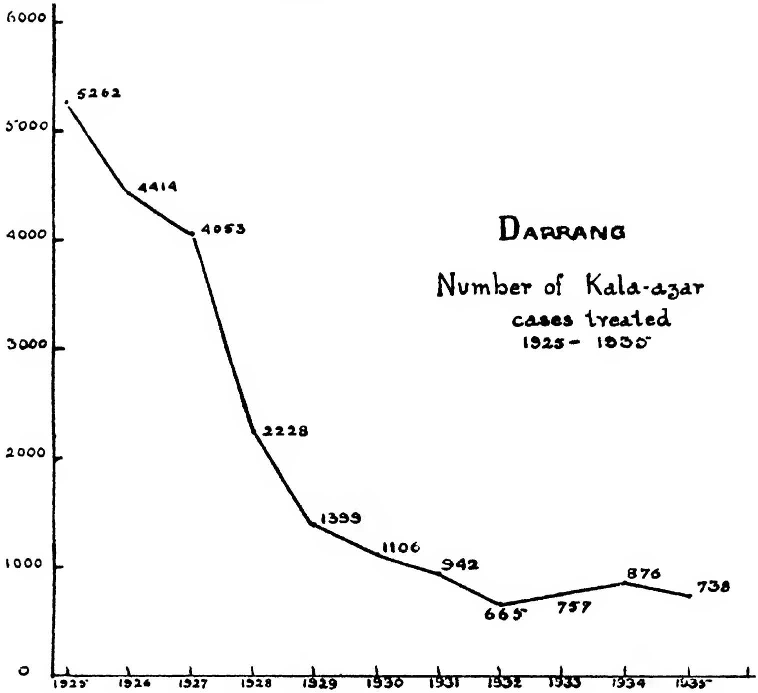
Darrang
Number of Kala-azar cases treated
1925 - 1935
6000
5000
4000
3000
2000
1000
0
5262
4414
4053
2228
1399
1106
942
665
757
876
738
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935

Fig. 9.

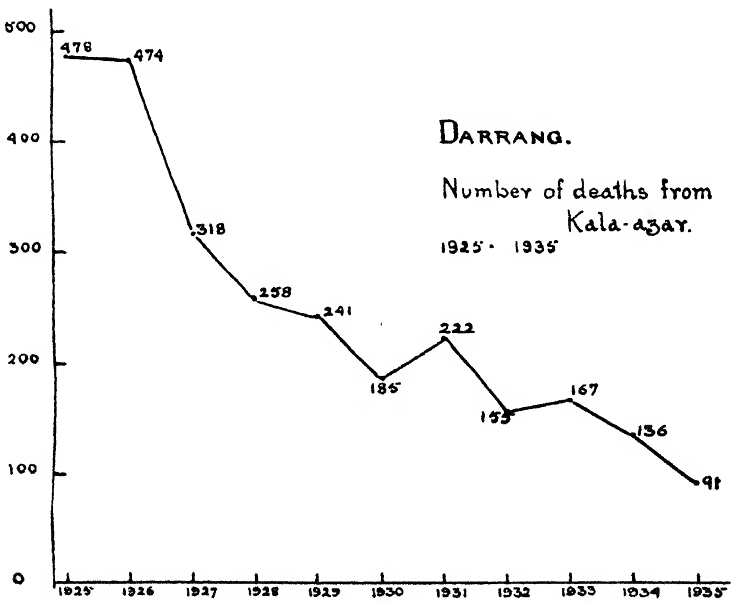
Darrang.
Number of deaths from Kala-azar.
1925 - 1935
500
400
300
200
100
0
478
474
318
258
241
185
222
155
167
136
91
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935

Fig. 10.

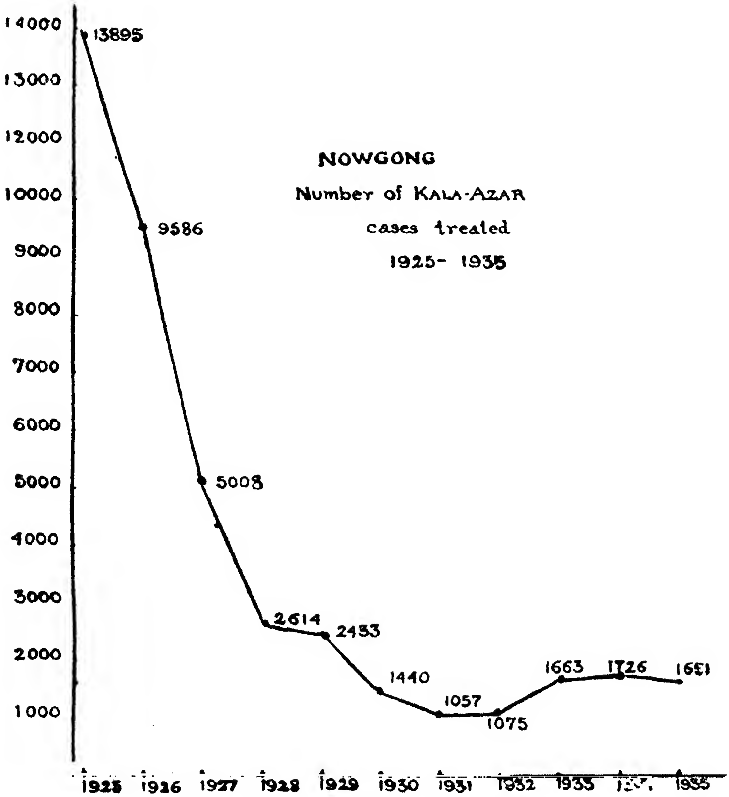
NOWGONG
Number of KALA-AZAR
cases treated
1925- 1935
14000
13000
12000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
13895
9586
5008
2614
2453
1440
1057
1075
1663
1726
1925
1926
1927
1928
1929
1930
1931
1932
1933
1935

FIG 11.

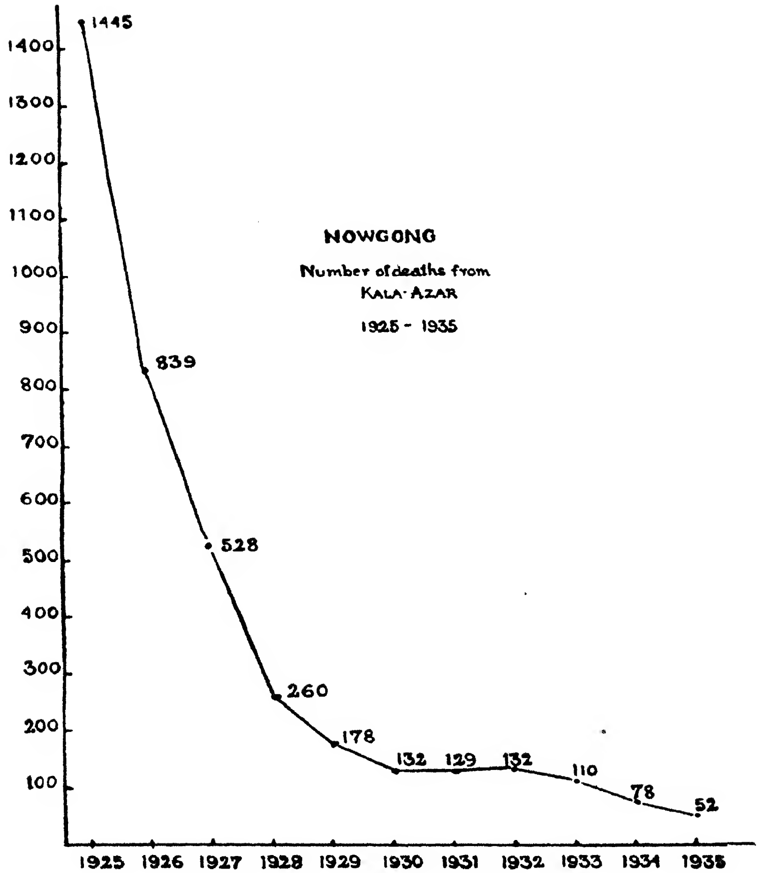
NOWGONG
Number of deaths from
KALA-AZAR
1925 - 1935
1445
839
528
260
178
132
129
132
110
78
52
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935

FIG. 12.

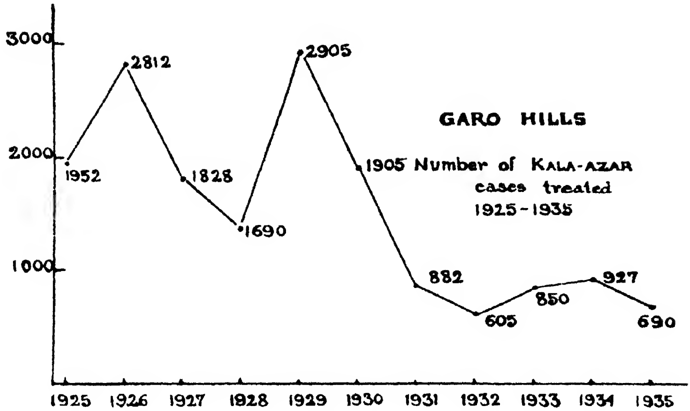
3000
2000
1000
2812
2905
1952
1828
1690
1905
882
605
850
927
690
GARO HILLS
Number of KALA-AZAR cases treated
1925-1935
1925 1926 1927 1928 1929 1930 1931 1932 1933 1934 1935

FIG. 13.

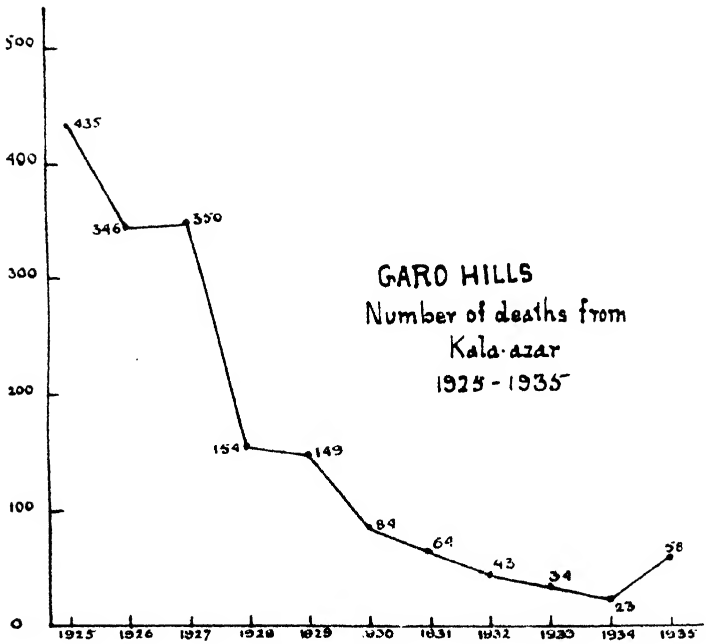
500
400
300
200
100
0
435
346
350
154
149
84
64
43
34
23
58
GARO HILLS
Number of deaths from
Kala-azar
1925-1935

FIG. 14.

STATISTICS OF THE NUMBER OF KALA-AZAR CASES TREATED BY THE GOVERNMENT OF ASSAM AND THE NUMBER OF DEATHS FROM THE DISEASE

PROVINCE OF ASSAM AS A WHOLE

| Year | 1925 | 1926 | 1927 | 1928 | 1929 | 1930 | 1931 | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of cases treated | 30,940 | 49,385 | 33,415 | 23,576 | 23,804 | 16,430 | 12,592 | 11,958 | 12,650 | 13,398 | 11,100 |
| No. of deaths ... | 6,365 | 4,176 | 2,859 | 1,660 | 1,405 | 953 | 1,017 | 987 | 749 | 770 | 845 |

DISTRICTS

*Sylhet*

| Year | 1925 | 1926 | 1927 | 1928 | 1929 | 1930 | 1931 | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of cases treated | 10,934 | 16,335 | 10,527 | 8,988 | 9,162 | 6,726 | 5,512 | 4,719 | 4,210 | 4.612 | 3,869 |
| No. of deaths ... | 2,109 | 1,320 | 798 | 482 | 429 | 274 | 246 | 296 | 146 | 227 | 260 |

*Goalpara*

| Year | 1925 | 1926 | 1927 | 1928 | 1929 | 1930 | 1931 | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of cases treated | 6,003 | 5,671 | 3,495 | 2,316 | 2,389 | 1,439 | 992 | 1,089 | 1,159 | 1,107 | 1,245 |
| No. of deaths ... | 453 | 297 | 226 | 166 | 135 | 112 | 121 | 122 | 92 | 61 | 100 |

*Kamrup*

| Year | 1925 | 1926 | 1927 | 1928 | 1929 | 1930 | 1931 | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of cases treated | 8,753 | 7,301 | 6,445 | 3,577 | 2,598 | 1,814 | 1,690 | 2,061 | 2,223 | 2,197 | 1,465 |
| No. of deaths ... | 1,120 | 714 | 475 | 241 | 180 | 102 | 160 | 152 | 129 | 151 | 176 |

*Darrang*

| Year | 1925 | 1926 | 1927 | 1928 | 1929 | 1930 | 1931 | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of cases treated | 5,262 | 4,414 | 4,053 | 2,228 | 1,399 | 1,106 | 942 | 665 | 757 | 876 | 738 |
| No. of deaths ... | 478 | 474 | 318 | 258 | 241 | 185 | 222 | 155 | 167 | 136 | 91 |

*Nowgong*

| Year | 1925 | 1926 | 1927 | 1928 | 1929 | 1930 | 1931 | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of cases treated | 13,895 | 9,586 | 5,008 | 2,614 | 2,433 | 1,440 | 1,057 | 1,075 | 1,663 | 1,726 | 1,651 |
| No. of deaths ... | 1,445 | 839 | 528 | 260 | 178 | 132 | 129 | 132 | 110 | 78 | 52 |

*Garo Hills*

| Year | 1925 | 1926 | 1927 | 1928 | 1929 | 1930 | 1931 | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of cases treated | 1,952 | 2,812 | 1,828 | 1,690 | 2,905 | 1,905 | 882 | 605 | 850 | 927 | 690 |
| No. of deaths ... | 435 | 346 | 350 | 154 | 149 | 84 | 64 | 43 | 34 | 23 | 58 |

The number of cases treated and of deaths in 1936 could not be incorporated in the above figures and tables, as the Annual Health Report for 1936 was not available when the figures and tables were printed off. Fig. 15 gives a comparative statement of the number of cases treated and deaths in the Province of Assam from 1925 to 1936.

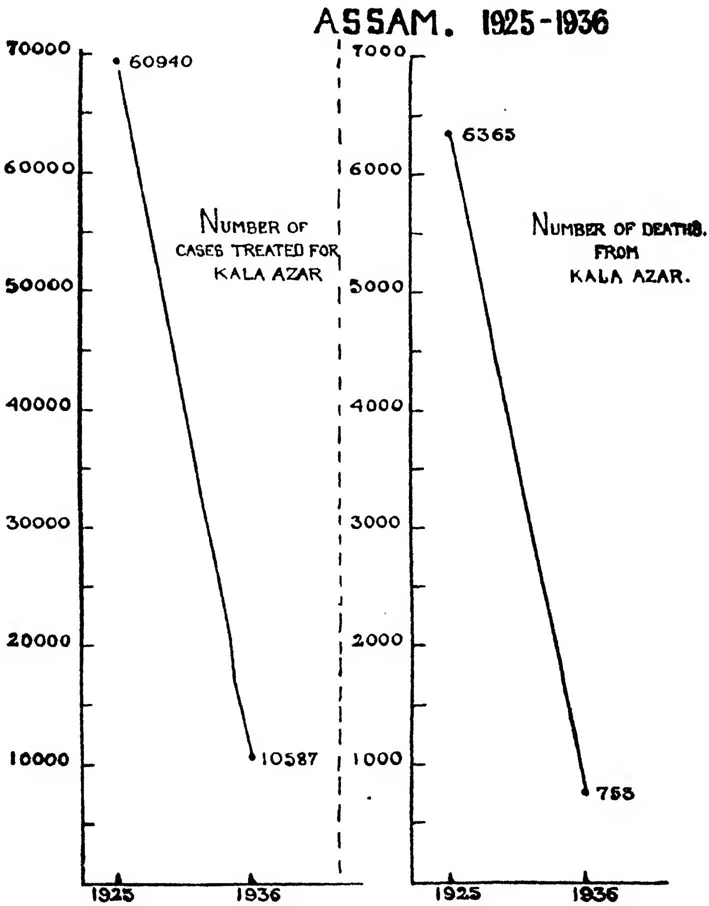


FIG. 15.

The figures for *Cachar* which are not exhibited in the above diagrams are very interesting. Out of 5,188 cases

treated from 1925 to 1936, the number of deaths was 56 showing a percentage of less than 1·08 per cent. Out of 574 cases treated in 1936 the number of deaths was 2 showing a percentage of less than 0·3 per cent.

It will be seen from the above figures and tables that the effect of the treatment with urea stibamine during the above-mentioned years on the incidence of kala-azar in Assam and its mortality has been phenomenal. The disease has lost all its terrors in the province and people who suffer from kala-azar are less afraid than those who suffer from malaria.

The Kala-azar Commission, India, used, throughout the seven years of its existence, urea stibamine only, in the routine treatment of kala-azar. According to them the acute fulminating type characteristic of the peak period of an epidemic responded to treatment with urea stibamine extraordinarily promptly and with an almost dramatic cessation of fever, diminution in size of the spleen and return to the normal condition of health. It may be expected that similar beneficial results will be obtained in other epidemics of the disease.

In 1933, the Director of Public Health, Assam, noted that ' urea stibamine was our mainstay in the treatment of kala-azar.' ' Since 1923, when reliable figures for the diseases first became available to the end of the year under report, no less than 328,591 persons have been brought under treatment. It is no exaggeration to say that approximately 3·25 lacs of valuable lives have been saved to the Province.'

Sir John Kerr, once Governor of Assam, in his farewell address to the Assam Legislative Council referring to the value of urea stibamine stated that ' the progress in the campaign against kala-azar in Assam has been phenomenally rapid and if it continues at the present rate there is an excellent prospect of the dread scourge being brought under complete control in a few years.' This has now come to

pass, as you have just seen from the statistics quoted in the present address, and as was once predicted by the Director of Public Health, Assam, one day we shall be successful in stamping the disease out of the province. The same may also be said of other parts of the world where the disease occurs. The discovery of a powerful specific for the disease, its limited distribution and rarity of relapses lend support to this conclusion.

A few antimonials have been tried intramuscularly in the treatment of kala-azar. Among these may be mentioned sodium-N-phenyl-glycine-amide-4-stibinate (antimony analogue of tryparsamide) and sodium-sulphomethylene-stibanilate (antimonyl analogue of sulph-arsenol) which have been successfully used by the author, while neostibosan has been used with success by Napier.

In studying the treatment of kala-azar one finds that the enemy in the process of destruction tries to retreat from the internal organs to some other parts of body, just as it is noticed in the case of human warfare the conquered foe tries to hide himself in hills and jungles to elude the pursuit of the conqueror. This was first discovered in 1922 when the speaker observed certain remarkable skin eruptions caused by *Leishmania donovani* developing in kala-azar patients two or three years after completion of antimonial treatment and apparent cure, though, under ordinary conditions, in kala-azar the skin shows very little involvement or none. Originally considered very rare, these skin lesions have been subsequently found not to be an uncommon condition.

The disease was named dermal leishmanoid by the speaker when first discovered, and subsequently called dermal leishmaniasis in the Carmichael Hospital for Tropical Diseases attached to the Calcutta School of Tropical Medicine.

The various types of the disease will not be described here in detail.

For the photograph of the first recorded case of dermal leishmanoid. See Plate LXIII, facing page 52.

The photograph of a case of annular variety of the disease and of the photomicrographs of sections of skin showing the presence of leishmania-laden cells just under the epidermal layer and of leishmania-laden pigment-carrying cells in the superficial layer of the dermis are reproduced here.

Viable leishmania have been cultured from these skin lesions in test tubes and sandflies. They are therefore a source of infection and the conquest of kala-azar cannot be regarded complete unless these lesions are either averted or quickly cured. Not infrequently, they require a prolonged course of antimonial treatment and some of them are very resistant and may be dangerous carriers of antimony-resistant parasites. The author has recommended combined treatment with intravenous injection of urea stibamine and inunction of metallic antimony.

It is evident that in the campaign against kala-azar and its conquest, proper handling of cases of dermal leishmanoid is an important point to be taken into consideration.

The constitution of urea stibamine has been a matter of some controversy. As pointed out by Gray and co-workers it is the most interesting of therapeutic antimonials. Originally considered by the speaker to be a urea salt of para-amino-phenyl stibinic acid, it was subsequently described by him to be ammonium carbamino-stibanilate. More recently Gray and co-workers have studied the chemical constitution and physiological properties of the compound carefully and exhaustively in an important paper in the *Proceedings of the Royal Society* (1931). They have shown that urea stibamine is disubstituted urea consisting mostly of S-diphenylcarbamide-4 : 4′-distibinic acid as its active organo-metallic constitution, containing some amount of protective colloids to make it water-soluble. Its constitution is therefore, according to these observers,

different from that of compounds of the type of neostibosan or neostam which are salts of para-aminophenyl-stibinic acid.

The conquest of kala-azar by campaign against the disease by treatment of the affected individuals is, from what I have shown, one of the most remarkable feats in chemotherapy. Whether a prophylactic dose of urea stibamine to persons living in kala-azar infected areas, just like the prophylactic use of quinine in malaria, will be of any value or whether an inunction of metallic antimony may be recommended to be used by them as a routine practice, is a matter for investigation.